Treatment of individuals at clinical high risk for psychosis

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Treatment of Individuals at Clinical High Risk for Psychosis

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For my Mum and Dad, who worked
tirelessly and selflessly throughout their lives
to give me the best possible start in mine.

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Finally, I would like to thank the participants who volunteered to take part, without whom this research would not have been possible.

ABSTRACT

People at Clinical High Risk for psychosis (CHR-P) present with a clinical syndrome that includes attenuated positive psychotic symptoms and impairments of social/emotional functioning. The CHR-P state is associated with a 20% risk of developing psychosis over a two-year period. However, treatment options remain limited—no licensed pharmacological therapies are currently available, and the comparative efficacy of the available treatments remains unknown. The purpose of this PhD was to address aspects of these outstanding issues in two distinct but complementary ways.

Part 1 of this thesis provides an evidence synthesis of the CHR-P treatment literature. Following a qualitative introductory review, I used systematic review and network meta-analyses to compare and summarise the relative efficacy and acceptability of current treatments for (a) preventing transition to psychosis from a CHR-P state **[Paper 1]**, and (b) reducing attenuated positive psychotic symptoms **[Paper 2]**. The results of Papers 1 and 2 indicated that, to date, there is a lack of evidence that any specific intervention is particularly effective over any others in preventing transition to psychosis from a CHR-P state or in reducing attenuated positive psychotic symptoms. These results also suggest a need to identify potential novel therapeutics that may better target the pathophysiological mechanisms underlying psychosis onset and that may thereby alter the course of the disorder.

Part 2 of this thesis sought to examine the neurophysiological basis for the effects of a potential novel treatment strategy, the neuropeptide oxytocin, in an intranasal oxytocin vs placebo acute challenge study using magnetic resonance imaging. Part 2 starts with the rationale for selecting oxytocin from the numerous candidate compounds indicated for those at CHR-P, followed by a discussion of the oxytocinergic system and its links to psychosis and the CHR-P state. I then present two experiments that tested whether oxytocin modulated (a) resting cerebral perfusion using arterial spin labelling, with a particular focus on the hippocampus **[Paper 3]**, and (b) neurochemical metabolite levels (particularly glutamate, and glutamate plus glutamine, Glx) in the hippocampus, thalamus, and anterior cingulate cortex, as measured using proton magnetic resonance spectroscopy **[Paper 4]**. The results of the two neuroimaging experiments suggested that oxytocin can modulate hippocampal perfusion in CHR-P individuals [Paper 3]—which is a key pathophysiological mechanism strongly implicated in psychosis onset—

but does not appear to have effects on glutamate or Glx concentrations in the hippocampus, thalamus, or anterior cingulate cortex [Paper 4]. However, based on the hippocampal perfusion findings alone, oxytocin merits further investigation as a candidate novel treatment for this group.

Part 3 of this thesis extends the discussion of the findings from Part 1 and Part 2. Here, I use the results of the evidence syntheses and wider literature to highlight a number of critical challenges, and proposed solutions, for future CHR-P treatment research. I then place the experimental oxytocin findings in the context of CHR-P pathophysiology, before bringing together the two distinct methodological approaches (evidence synthesis and experimental medicine) to show how both—combined—could facilitate a new era of improved interventional research in the CHR-P field.

STATEMENT OF PERSONAL CONTRIBUTION

I executed all components of the two network meta-analyses, including the analyses and writing of the first drafts of the manuscripts. For the MRI study, I made amendments to the ethical approval to add new recruitment sites, I led and conducted the recruitment (with support of study clinicians), I conducted and organised the data collection, independently conducted all data management and cleaning, performed all data analyses and wrote the first drafts of the manuscripts. Finally, I wrote this thesis in its entirety, with the following exception: the four published papers, after being drafted by me, were circulated to co-authors and underwent peer review prior to acceptance, leading to editing of the manuscripts.

PREFACE

This thesis is a "thesis incorporating publications". This refers to the fact that a number of chapters are composed of published journal articles of which I am the first author.

Publications relating to the work presented in this thesis – four chapters are composed of the following journal articles which are reproduced in full:

- Chapter 2, Paper 1 – Network Meta-Analysis for Transition (published) (Davies *et al*, 2018a)
- Chapter 3, Paper 2 – Network Meta-Analysis for Attenuated Psychotic Symptoms (published) (Davies *et al*, 2018b)
- Chapter 5, Paper 3 – Oxytocin, Arterial Spin Labelling Study (published) (Davies *et al*, 2019a)
- Chapter 6, Paper 4 – Oxytocin, Proton Magnetic Resonance Spectroscopy Study (in press) (Davies *et al*, 2019b)

In addition, the general discussion section [Part 3 of this thesis] contains text from the following paper which was co-authored by me and largely based on my work in Papers 1 and 2:

- [Paper 5] – Fusar-Poli P, Davies C, Solmi M, Brondino N, Kotlicka-Antczak M, Shin J, Radua J. Preventive treatments for psychosis: umbrella review (just the evidence). *Frontiers in Psychiatry* (under review).

NB: In places within this thesis, I use "our" and "we" when referring to the published research for consistency with the four included manuscripts.

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LIST OF ABBREVIATIONS

1H-MRS – Proton Magnetic Resonance Spectroscopy

ACC – Anterior Cingulate Cortex

ASL – Arterial Spin Labelling

CAARMS – Comprehensive Assessment of At-Risk Mental States

CBF – Cerebral Blood Flow

CBT – Cognitive Behavioural Therapy

CHR-P – Clinical High Risk for Psychosis

CI – Confidence Intervals

Cr – Creatine

CRLB – Cramer-Rao Lower Bounds

CSF – Cerebrospinal Fluid

DSM – Diagnostic and Statistical Manual of Mental Disorders

FEP – First Episode Psychosis

FWHM – Full-Width at Half-Maximum

FWE – Family-Wise Error

fMRI – functional Magnetic Resonance Imaging

GABA – Gamma-Aminobutyric acid

Gln – Glutamine

Glu – Glutamate

Glx – Glutamate plus Glutamine

ICD – World Health Organisation International Classification of Diseases

mPFC – Medial Prefrontal Cortex

MRI – Magnetic Resonance Imaging

mM – millimolar

ms – milliseconds

NAA – N-Acetyl-Aspartate

nM – nanomolar

NMDA – N-Methyl-D-Aspartate

NMDAR – N-Methyl-D-Aspartate Receptor

PANSS – Positive and Negative Syndrome Scale

PRESS – Point RESolved Spectroscopy

rCBF – resting state regional Cerebral Blood Flow

RCT – Randomised Controlled Trial

RM-ANCOVA – Repeated Measures Analysis of Covariance

SD – Standard Deviation

SE – Standard Error

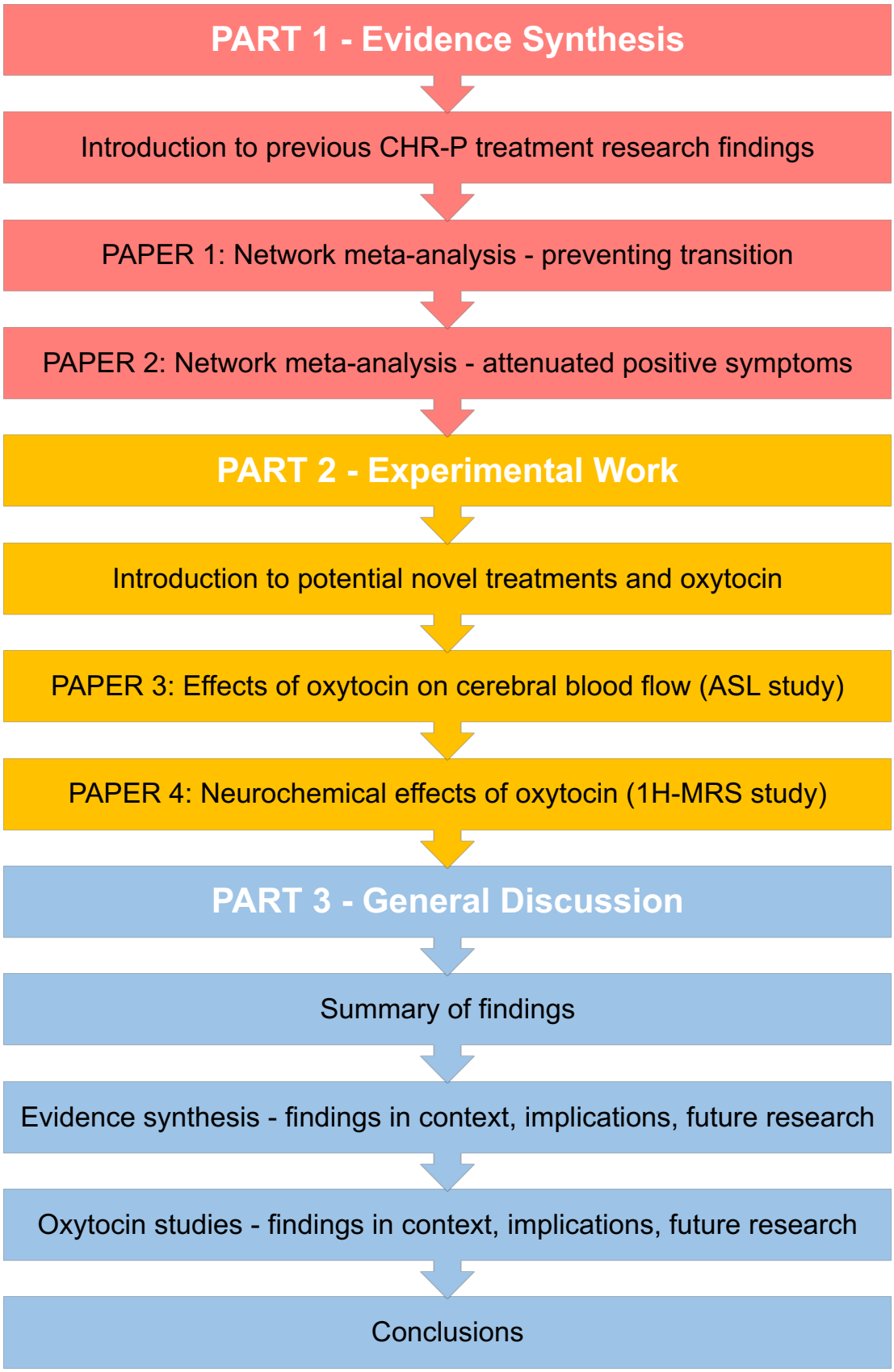
SIPS – Structured Interview for Prodromal Symptoms

SNR – Signal-to-Noise Ratio

STAI – State-Trait Anxiety Inventory

UHR – Ultra-High Risk for Psychosis

THESIS OUTLINE



PART 1 – TREATMENTS FOR THE CHR-P STATE: EVIDENCE SYNTHESIS

1. INTRODUCTION

1.1. THE CHR-P STATE

The Clinical High Risk for Psychosis (hereafter CHR-P) construct was introduced in 1995 to enable the prospective identification of individuals at incipient risk for psychotic disorders (Yung *et al*, 1996, 2005), which opened up new possibilities for research into preventative treatments. As such, preventative intervention within this putative prodrome became not only feasible but a clinical priority. Individuals at CHR-P present with attenuated positive psychotic symptoms, cognitive (basic) symptoms, impaired social and emotional function, neurophysiological alterations (at group level) and help-seeking behaviour (Fusar-Poli *et al*, 2013a). Whether a patient meets CHR-P criteria is established using validated semi-structured interviews such as the Comprehensive Assessment of At-Risk Mental States (CAARMS) (Yung *et al*, 2005) and Structured Interview for Prodromal Syndromes (SIPS) (Miller *et al*, 2003). Over recent years, the risk of developing psychosis in CHR-P samples has declined from approximately 30% (Fusar-Poli *et al*, 2012a) to 20% (Fusar-Poli *et al*, 2016b) at two years, although there are some exceptions (Fusar-Poli *et al*, 2018c). Preventing transition (or "progression") to psychosis from a CHR-P state has been a key goal of this paradigm and has been the primary outcome in the majority of treatment research studies to date (Fusar-Poli *et al*, 2013a). This follows the conceptualisation of the CHR-P state as a putative prodrome, in which there is an (unique) opportunity to arrest early pathophysiological processes before more severe or enduring neural changes take place (Millan *et al*, 2016). Further goals include reduction of attenuated positive and negative symptoms, improved social and occupational functioning and quality of life.

With accumulating knowledge has come increased focus on clinical services for CHR-P individuals, with specialist CHR-P clinics now available in many countries worldwide. In the UK, the specialist assessment, treatment and monitoring of CHR-P individuals is recognised as a key component of secondary mental health services by the National Institute for Health and Care Excellence (NICE, 2014) guidelines (Guideline CG178). However, as with medicine more broadly, if the CHR-P construct —which is essentially prognosis (Fusar-Poli *et al*, 2018b)—is to be useful and provide tangible benefits for patients, we need effective interventions. While results of previous treatment studies have been invariably mixed and often conflicting, the current NICE guidelines recommend that individual Cognitive Behavioural Therapy (CBT) be offered, with or without family interventions, while antipsychotics are prohibited (NICE, 2014).

Currently, there are no licensed pharmacological treatments for this patient group, which remains an unmet clinical need.

1.2. CHR-P TREATMENT RESEARCH

Over the past two decades, numerous psychological and pharmacological interventions have been tested in randomised controlled trials (RCTs), with varying levels of success. This qualitative introduction aims to provide an overarching perspective of this literature, with a particular focus on two key outcomes: preventing transition to first-episode psychosis from a CHR-P state and reducing attenuated positive psychotic symptoms.

1.3. PHARMACOLOGICAL AND COMBINED TREATMENTS

1.3.1. ANTIPSYCHOTICS

Risperidone and CBT

The first randomised, double-blind, placebo-controlled interventional trial in the CHR-P field took a "best bet" approach, combining risperidone (mean dose = 1.3mg/day) and individual CBT and comparing this package to a needs-based intervention (total N=59) (McGorry *et al*, 2002). After 6 months of treatment, 10 of the 28 individuals receiving needs-based interventions had transitioned vs 3 of 31 receiving risperidone and CBT (P=.03). However, by 12 months and using intention-to-treat analysis, there was no significant difference between treatment arms. One limitation of this trial was that the relative contributions of risperidone and CBT could not be separated. In a later, larger (N=115) multi-arm RCT, risperidone plus CBT was compared to placebo plus CBT, and placebo plus supportive therapy (McGorry *et al*, 2013; Yung *et al*, 2011). Here, after 12 months of treatment, no significant differences in transition risk emerged between the three treatment arms (with transition risk at approximately 10% in each group, which is relatively low).

Olanzapine

One of the earliest interventional RCTs used 5-15mg/day olanzapine in a fixed-flexible dosing schedule (Woods *et al*, 2003), finding a significant reduction in attenuated psychotic symptoms in the olanzapine vs placebo group after 8 weeks of treatment, although this was accompanied by significantly greater weight gain. However, the longer-term follow up of the study found no significant difference in transition to psychosis, or in overall severity of attenuated psychotic symptoms (McGlashan *et al*, 2006), although the olanzapine group did show significantly lower levels of symptoms

between weeks 8–28 of the treatment year.

Aripiprazole

Aripiprazole has been tested in two studies. The first used a fixed-flexible dosing schedule (5-30mg/day) for eight weeks in a small (N=15), uncontrolled, open-label pilot study (Woods *et al*, 2007). No participants transitioned to psychosis (albeit at 8 weeks, the study duration was short) and a significant reduction in attenuated psychotic symptoms was observed after one week of treatment and at all further timepoints. A large, multi-arm, double-blind RCT comparing aripiprazole, placebo, and CBT has since been conducted; the initial results appear to show no significant difference in the number of transitions between treatment arms (Bechdolf *et al*, 2016) but the full scientific report is yet to be published.

Ziprasidone

More recently, ziprasidone (20-160mg/day) was compared to placebo in a randomised, double-blind design (N=51) (Woods, 2016). There was no significant difference in the number of individuals transitioning to psychosis (likely owing to a lack of power for assessing this outcome), but a significant difference favouring ziprasidone was observed in levels of attenuated psychotic symptoms (Woods *et al*, 2017). There were no group differences in weight gain or QTc prolongation, potentially suggesting a more favourable side-effect profile relative to other antipsychotics, such as olanzapine.

Amisulpride

A randomised, open-label study (N=124) of amisulpride (50-800mg/day) vs placebo (both combined with needs-based intervention) found that amisulpride produced superior effects on attenuated and full-blown psychotic symptoms, global functioning and depressive, negative and basic symptoms (Ruhrmann *et al*, 2007). Transition was not assessed.

Perospirone

Finally, one very small (N=11), uncontrolled, open-label study of perospirone reported significant improvements in total and attenuated positive symptom scores (measured using the Scale of Prodromal Symptoms, SOPS) from baseline to 26 week follow up (Tsujino *et al*, 2013).

1.3.2. ANTIDEPRESSANTS

A naturalistic study of interventions delivered in real-world clinical routine demonstrated that antidepressants plus CBT were associated with lower transition risk over time relative to antipsychotics plus CBT (Hazard Ratio (HR)= 0.129, 95% CI 0.03–0.57, P=.007), which did not appear to be secondary to different baseline levels of psychopathology (Fusar-Poli *et al*, 2015b). Another naturalistic study found significantly higher transition risk in antipsychotic vs antidepressant treated patients, although nearly all transitions were associated with antipsychotic nonadherence (Cornblatt *et al*, 2007). While these and other studies (Cannon *et al*, 2008; Kim *et al*, 2012) may suggest a therapeutic effect of antidepressants on transition, there are numerous limiting factors associated with these studies. The naturalistic design means that a causal link between antidepressant prescription and psychosis prevention cannot be made. For example, there appeared to be a preponderance of patients belonging to the Brief Limited Intermittent Psychotic Symptoms (BLIPs) subgroup—who have the highest transition risk of all subgroups (Fusar-Poli *et al*, 2016b)—in the antipsychotic-treated group, and a trend-level greater number of affective comorbidities in the antidepressant-treated group (Fusar-Poli *et al*, 2015b). This may indicate that differences in baseline psychopathology and clinical presentation were subtly influencing clinicians' treatment decisions, meaning that those with the highest levels of risk (such as the BLIPs) were prescribed antipsychotics, and those with lowest risk (e.g. those false-positives who will later go on to develop non-psychotic disorders or remit) were prescribed antidepressants.

1.3.3. EXPERIMENTAL TREATMENTS

Omega-3

Following positive results in patients with schizophrenia (Irving *et al*, 2006) and given the known, potentially serious adverse effects associated with antipsychotic medication, there was great optimism that long-chain omega-3 polyunsaturated fatty acid supplementation might also prove efficacious in CHR-P populations. In the first study of its kind, 81 individuals were randomised to receive either 1.2g/day omega-3 or placebo over 12 weeks followed by a monitoring period (Amminger *et al*, 2010). Omega-3 was found to significantly reduce the risk of transition, with 5% of the omega-3 group and 28% of the placebo group having transitioned to psychosis by 12 months (P=.007). Relative to placebo, omega-3 also significantly reduced positive and negative symptoms and improved functioning. These beneficial effects on transition risk and functioning appeared to have been maintained long-term, until approximately 6.7 years

of follow up (Amminger *et al*, 2015). However, in the largest of all RCTs in this field to date (N=304), omega-3 (1.4g/day) was compared to placebo, both combined with cognitive-behavioural case management over 6 months (McGorry *et al*, 2017). Here, no significant differences in transition risk or symptoms were observed at 6 or 12 months, with the medium-term follow up (mean 3.4 years) recently reaching a similar conclusion (Nelson *et al*, 2018a). The authors suggest that the high quality psychosocial intervention delivered in (both arms of) this trial may have produced a ceiling effect, beyond which omega-3 had little opportunity to demonstrate a significant benefit (McGorry *et al*, 2017). These sobering findings were echoed again in an independent study of 127 patients from the NAPLS consortium, where omega-3 failed to provide any benefit on risk of transition relative to placebo (Cadenhead *et al*, 2017). As is the case in the majority of studies in CHR-P individuals, both groups significantly improved in terms of symptoms and functioning over time but there were no between-group differences. This also demonstrates why uncontrolled studies in this patient population are problematic and must be interpreted with caution—even in the absence (or regardless) of treatment, these patients often improve from baseline over time (Fusar-Poli *et al*, 2015b).

Glycine site compounds

Three studies have tested glycine site allosteric modulators (co-agonists) of the N-methyl-D-aspartate (NMDA) receptor, namely D-serine and glycine. In a pair of related, small-scale pilot studies, oral glycine was first titrated (open-label) in 10 CHR-P individuals for 8 weeks (Woods *et al*, 2013). Significant within-group improvements in total, positive, negative and depressive symptoms were observed. However, the authors then conducted a randomised, double-blind, glycine vs placebo study in 8 patients for 12 weeks (Woods *et al*, 2013), finding a significant reduction in depressive symptoms only. Transition was not explicitly investigated due to the short study duration. The extremely small sample sizes in these studies was a clear and major limitation, but they did provide initial proof-of-concept evidence. Shortly after, in a randomised, double-blind, placebo-controlled study, 44 CHR-P individuals received ~4.2g/day of D-serine or placebo for 16 weeks (Kantrowitz *et al*, 2015). D-serine led to a significant (36%, SD=18) reduction in negative symptoms (P=.03), which was the study's primary outcome. There were no effects on attenuated positive or total symptoms as measured using the SOPS and there were too few transitions to test this outcome.

N-acetylcysteine

Based on its antioxidant and neuroprotective profile of effects, n-acetylcysteine, a glutathione precursor, was tested in a very small uncontrolled case series of 5 CHR-P individuals for 12 weeks (Miyake *et al*, 2016). There were no significant within-group effects on total SOPS scores, but n-acetylcysteine appeared to be well tolerated and potential beneficial effects on cognitive functioning were observed at 24-week follow up. However, the small case series design means that these results are unlikely to be robust. A large, multi-centre RCT of n-acetylcysteine is now underway (Schmidt *et al*, 2019).

1.4. PSYCHOLOGICAL TREATMENTS

1.4.1. COGNITIVE BEHAVIOURAL THERAPY (CBT)

Individual CBT is the most studied intervention for CHR-P individuals. While the exact protocols differ between studies, broadly speaking, 6 RCTs have been conducted to date. The first study, from 2004, compared 6 months of CBT to treatment as usual (usually defined as "needs-based interventions") in 58 CHR-P individuals (Morrison *et al*, 2004). CBT significantly reduced transition to psychosis over a 12-month period (odds ratio (OR)=0.04, 95% CI 0.01–0.71, P=.028) as well as the severity of attenuated psychotic symptoms. However, blinding was not maintained and when an intention-to-treat approach was applied, there were no significant results. The second RCT compared 6 months of CBT to supportive therapy in 51 CHR-P patients, and found no significant differences in transition, attenuated positive and negative symptoms, depression, anxiety or social functioning as assessed at 6, 12 and 18-month follow ups (Addington *et al*, 2011). These results were echoed by a subsequent, larger study (N=288), where CBT plus monitoring was compared to monitoring only (Morrison *et al*, 2012). Here, no effects on transition or symptom-related distress were observed, but the severity of attenuated psychotic symptoms was significantly reduced at 12 months in the CBT group. The low transition risk (7% in CBT group, 9% in monitoring group) may have led to underpowered analyses for the transition outcome (Morrison *et al*, 2012). Using an enhanced CBT protocol that specifically targeted cognitive biases and psychoeducation on dopamine system super-sensitivity, one study randomised 201 patients to CBT or treatment as usual for 6 months, with an 18-month follow up (van der Gaag *et al*, 2012). This CBT protocol significantly reduced transition to psychosis (OR=0.52, 95% CI 0.19–0.95) as well as the distress associated with attenuated psychotic symptoms at 6 months. The overall transition risk of 16.3% is notably higher than the aforementioned studies that found no treatment effects. However, it should be

noted that using an intention-to-treat approach abolished the significant results, and for the distress outcome, data were based on non-transitions only (van der Gaag *et al*, 2012). Contrasting results were found in a more recent RCT of 57 CHR-P individuals, which compared CBT to a more "active" control condition (non-directive reflective listening), both in addition to standard care (Stain *et al*, 2016). There were too few transitions to perform statistical analyses on this outcome (5% transition; 3 transitions in the CBT group and 0 in the control group), but the control treatment significantly reduced the distress associated with attenuated psychotic symptoms relative to CBT. Compared to the other trials, the sample in this study was younger, higher functioning and had lower levels of attenuated psychotic symptoms. Such characteristics may account for the better treatment effects of the control intervention and would be in line with the clinical staging model (Stain *et al*, 2016). As previously mentioned, initial results from a large (N=280), multi-arm, single-blind RCT comparing aripiprazole, placebo, and CBT appeared to show no difference between any treatment arms in preventing transition, although the full report has yet to be published (Bechdolf *et al*, 2016).

1.4.2. INTEGRATED PSYCHOLOGICAL INTERVENTIONS (IPI)

Individual CBT has also been tested as part of a multi-component package of therapies, which included group skills training, cognitive remediation and multifamily psychoeducation, termed "integrated psychological interventions", which was compared to supportive counselling, both delivered for 12 months, in 128 individuals (Bechdolf *et al*, 2012). The results showed that integrated psychological interventions significantly reduced the number of transitions at 12 (3.2% vs 16.9%, P=.008) and 24-month (6.3% vs 20.0%, P=.019) follow ups.

1.4.3. FAMILY-FOCUSED THERAPY (FFT)

Family therapy is suggested as a treatment option for CHR-P patients in the current NICE guidelines (NICE, 2014). However, only one RCT (N=129) has investigated family-focused therapy vs an "enhanced care" control condition in these individuals (Miklowitz *et al*, 2014). Here, the family intervention was associated with greater improvement in attenuated psychotic symptoms over 6 months relative to control. There were fewer transitions in the family-focused group (2% vs 11%), although this was not statistically tested. The overall risk of transition was 6%. A different group-and-family-based CBT intervention has also been tested in one small (N=6), open, uncontrolled study, but the methodological shortcomings from the poor design limit its interpretation

(Landa *et al*, 2016).

1.4.4. OTHER STUDIES

One study took a risk-based allocation approach, assigning those with the highest risk of psychosis to a family-aided assertive community treatment, while those with lower risk were assigned to community care (McFarlane *et al*, 2015). The family-aided treatment demonstrated some benefits over community care, but no difference was observed in transition to psychosis and the quasi-experimental design severely hampers interpretation of the results. A separate study, that has often been included in reviews and meta-analyses of CHR-P patients, focused on patients with schizotypal disorder, finding that an integrated treatment (including social skills training and multi-family group psychoeducation) significantly reduced risk of transition relative to standard care (Nordentoft *et al*, 2006). However, strictly speaking, schizotypal disorder is not *per se* comparable to the CHR-P state (genetic risk and deterioration syndrome includes schizotypal disorder plus functional decline in help-seeking patients) as defined by consensus criteria (Fusar-Poli *et al*, 2013a). Finally, a number of studies of cognitive remediation have been conducted but they have not focused on preventing transition to psychosis (Hooker *et al*, 2014; Loewy *et al*, 2016; Piskulic *et al*, 2015; Urben *et al*, 2012). However, a large RCT in CHR-P individuals that will assess effects on symptoms is currently underway (Glenthøj *et al*, 2015).

From this qualitative review, it becomes clear that many trials provide conflicting results regarding the efficacy of their tested treatments. In an attempt to overcome such discrepancies, a number of research groups have performed meta-analyses. In the following section, a review of these meta-analyses, their results and associated methodological concerns and limitations are discussed.

1.5. PREVIOUS SYSTEMATIC REVIEWS AND META-ANALYSES

One of the first comprehensive meta-analyses was conducted by Stafford *et al* in 2013, who performed a series of pairwise meta-analyses of different treatments and stratified results by time (Stafford *et al*, 2013a, 2013b), which is important given the time-varying outcomes in this patient group (Fusar-Poli *et al*, 2016b; Kempton *et al*, 2015). At 6 months, CBT did not reduce transition to psychosis relative to supportive counselling (risk ratio (RR)=0.62, 95% CI 0.29–1.31; 4 studies), but in those completing treatment, CBT was associated with a significant reduction in transition at 12 months (RR=0.54, 95% CI 0.34–0.86; 5 studies) and beyond 12 months (RR=0.63, 95% CI 0.40–0.99; 4

studies). While there was no significant meta-analytic benefit of CBT on attenuated positive symptoms at 6–12 months, the multiple errors in reporting these results (see figure 4 and the erratum in (Stafford *et al*, 2013a, 2013b)) prevents citation of the exact statistics. The only other treatment comparisons with more than one study available for meta-analysis was CBT plus risperidone vs supportive counselling, with CBT plus risperidone associated with significantly reduced transition at 6 months (RR=0.35, 95% CI 0.13–0.95; 2 studies) but not at 12 months (RR=0.63, 95% CI 0.33–1.21; 2 studies). Overall, the authors found mostly low to very low quality evidence, but their suggestion for treatment strategies combining family and individual CBT appear to have had a major influence on the current NICE guidelines, which recommend these interventions (NICE, 2014).

Another meta-analysis, from the same year, did not stratify their analyses by specific treatments at all, instead pooling all of the different interventions (including olanzapine, omega-3, CBT, integrated psychological interventions, risperidone plus CBT) together and comparing them to the various control conditions (van der Gaag *et al*, 2013). At 12 months, they found that the treatment conditions generally designated as the "active" intervention of interest, when pooled into a heterogeneous group (including olanzapine, omega-3, CBT, integrated psychological interventions, risperidone plus CBT), were significantly more efficacious in reducing transition relative to those treatment conditions generally labelled "control" (RR=0.46, 95% CI 0.33–0.64). This convoluted finding serves to highlight why such an approach is not a useful or clinically meaningful evidence synthesis approach; these results do not tell us how effective any of the aforementioned treatments are (at the meta-analytic level) beyond the results of the individual studies themselves, thereby defeating the purpose of the meta-analysis. In addition, one study that they included was a study of schizotypy rather than CHR-P individuals (Nordentoft *et al*, 2006). The authors then go on to present an analysis by "type of intervention", which raises further problems. They find that combining heterogeneous treatments that include any antipsychotic significantly reduced transition (RR=0.55, P=.029), but two of the three studies were of antipsychotics plus CBT. Furthermore, what the authors do not make explicitly clear is that their meta-analysis of CBT-based interventions at 12 months showed no significant benefit of CBT over control (RR=0.52, 95% CI 0–79). This point is obfuscated within the abstract and article text and these data are not presented in any of the forest plots, which the incorrect labelling of their figure 2 (which labels the plot as 'CBT' vs 'treatment as usual', when

it is in fact ‘all different treatments’ vs ‘all different controls’), misleadingly claims to do (van der Gaag *et al*, 2013).

A more recent meta-analysis, conducted for the European Psychiatric Association (EPA) guidelines, similarly asked (a) whether there is evidence that “focused early interventions” (as a whole, combining—as previously discussed—all different treatment types) are effective compared to controls, and (b) whether there are differences between two broad classes of interventions: any pharmacological and any psychological (Schmidt *et al*, 2015). Although they find a significant benefit of the more “focused” treatments in reducing transition at all time points (with only fixed effect results reported, when random effects would be more appropriate), because the authors did not stratify the comparisons by treatment, the EPA meta-analysis could not provide evidence on the likely effectiveness of any specific treatments, and again the problem of a broad-brush dichotomous approach (pharmacological vs psychological) is not specific enough to inform evidence-based clinical guidelines (Schmidt *et al*, 2015).

A 2014 meta-analysis focusing specifically on CBT (Hutton and Taylor, 2014) found no significant benefit of CBT on transition at 6 months (RR=0.52, 95% CI 0.27–1.02, 6 studies) but a significant effect emerged at 12 months (RR=0.45, 95% CI 0.28–0.73, 6 studies) and 18–24 months, albeit within this analysis they included the study of integrated psychological interventions which combines numerous approaches beyond CBT (Bechdolf *et al*, 2012). Similarly, there was no effect on (overall) symptoms at 6 months (Hedges’ g = -0.11, 95% CI -0.29 to 0.07, P=.23, 4 studies) but a significant benefit of CBT over control was found at 12 months (Hedges’ g = -0.25, 95% CI -0.46 to -0.03, P=.024, 5 studies) (Hutton and Taylor, 2014).

A number of additional (earlier) meta-analyses have been conducted but the limited number of included studies (Marshall and Rathbone, 2011; Preti and Cella, 2010) or very poor meta-analytic approach (Deas *et al*, 2016; Kelly *et al*, 2010) means that they provide little further insight to the results discussed above.

1.6. SUMMARY & STUDY RATIONALE

In summary, studies to date have investigated a variety of psychological and pharmacological interventions for those at CHR-P, including CBT, cognitive remediation, family-focused therapy, integrated psychological interventions, antipsychotics, antidepressants, omega-3, glycine-site compounds, and combined

interventions. However, many of these have been small, uncontrolled studies (Tsujino *et al*, 2013; Woods *et al*, 2007), some of low quality (Landa *et al*, 2016), while others were observational (Fusar-Poli et al., 2015). Across the randomised controlled trials, results have been markedly inconsistent. The three largest interventional studies in CHR-P individuals have all yielded negative findings (Fusar-Poli, 2017a; McFarlane *et al*, 2015; McGorry *et al*, 2017; Morrison *et al*, 2012).

Several pairwise meta-analyses of this topic have been conducted previously (van der Gaag *et al*, 2013; Hutton and Taylor, 2014; Preti and Cella, 2010; Schmidt *et al*, 2015; Stafford *et al*, 2013a), with the most consistent finding being of an approximate 50% reduction in transition risk associated with CBT compared to control interventions at 12 months but not at 6 months (and sometimes in later time points) (Hutton and Taylor, 2014; Stafford *et al*, 2013a, 2013b). Despite this, the evidence base for CBT's effectiveness appears far from robust. The largest CBT trial ever conducted in these patients showed no benefit of CBT over monitoring of mental state only (Morrison *et al*, 2012). In addition, previous meta-analytic results have often been estimated after pooling heterogeneous treatments together in pairwise comparisons (e.g. including integrated psychological interventions with studies of CBT alone (Hutton and Taylor, 2014)). The most recent meta-analysis without methodological shortcomings was conducted in 2013 (Stafford *et al*, 2013a, 2013b), and 7 new trials have since been conducted. Importantly, previous meta-analyses do not tell us which interventions are likely to be the most effective nor how each treatment fares against each other currently available treatment option. This leaves the possibility that a treatment other than CBT may be more effective.

In the following chapters (Papers 1 and 2), I address these issues by conducting an updated systematic review of the CHR-P treatment literature and performing a novel and sophisticated evidence synthesis approach: network meta-analysis. This method allows comparison of each treatment vs every other treatment on any available given outcome, is more statistically powerful than pairwise approaches, and allows the ranking of treatments from best to worst (Cipriani *et al*, 2013; Kanters *et al*, 2016). Specifically, in **Paper 1**, I present a published paper focusing on the efficacy and acceptability of treatments for preventing transition to psychosis. In **Paper 2**, I use the same methods but focus instead on the efficacy and acceptability of treatments for reducing attenuated (positive) psychotic symptoms. This is followed by an interim summary to conclude Part 1 of this thesis.

Aims and objectives

Papers 1 and 2 aim to:

- Quantify the consistency and magnitude of the efficacy of each specific intervention *for preventing transition to psychosis* in those at CHR-P using network meta-analysis (**Paper 1**).

- Quantify the consistency and magnitude of the efficacy of each specific intervention *for reducing attenuated positive psychotic symptoms* in those at CHR-P using network meta-analysis (**Paper 2**).

2. NETWORK META-ANALYSIS – TRANSITION

2.1. PAPER 1 – NETWORK META-ANALYSIS – TRANSITION

Davies C, Cipriani A, Ioannidis JPA, Radua J, Stahl D, Provenzani U, et al (2018). Lack of evidence to favor specific preventive interventions in psychosis: a network meta-analysis. *World Psychiatry* 17:196–209. https://doi.org/10.1002/wps.20526

RESEARCH REPORT

Lack of evidence to favor specific preventive interventions in psychosis: a network meta-analysis

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Preventing psychosis in patients at clinical high risk may be a promising avenue for pre-emptively ameliorating outcomes of the most severe psychiatric disorder. However, information on how each preventive intervention fares against other currently available treatment options remains unavailable. The aim of the current study was to quantify the consistency and magnitude of effects of specific preventive interventions for psychosis, comparing different treatments in a network meta-analysis. PsycINFO, Web of Science, Cochrane Central Register of Controlled Trials, and unpublished/grey literature were searched up to July 18, 2017, to identify randomized controlled trials conducted in individuals at clinical high risk for psychosis, comparing different types of intervention and reporting transition to psychosis. Two reviewers independently extracted data. Data were synthesized using network meta-analyses. The primary outcome was transition to psychosis at different time points and the secondary outcome was treatment acceptability (dropout due to any cause). Effect sizes were reported as odds ratios and 95% confidence intervals (CIs). Sixteen studies (2,035 patients, 57% male, mean age 20.1 years) reported on risk of transition. The treatments tested were needs-based interventions (NBI); omega-3 + NBI; ziprasidone + NBI; olanzapine + NBI; aripiprazole + NBI; integrated psychological interventions; family therapy + NBI; D-serine + NBI; cognitive behavioural therapy, French & Morrison protocol (CBT-F) + NBI; CBT-F + risperidone + NBI; and cognitive behavioural therapy, van der Gaag protocol (CBT-V) + CBT-F + NBI. The network meta-analysis showed no evidence of significantly superior efficacy of any one intervention over the others at 6 and 12 months (insufficient data were available after 12 months). Similarly, there was no evidence for intervention differences in acceptability at either time point. Tests for inconsistency were non-significant and sensitivity analyses controlling for different clustering of interventions and biases did not materially affect the interpretation of the results. In summary, this study indicates that, to date, there is no evidence that any specific intervention is particularly effective over the others in preventing transition to psychosis. Further experimental research is needed.

Key words: Psychosis, risk, prevention, needs-based interventions, cognitive behavioural therapy, antipsychotics, omega-3, integrated psychological interventions, family therapy, network meta-analysis, guidelines

(World Psychiatry 2018;17:196–209)

Individuals at clinical high risk for psychosis (CHR-P)[1] present with attenuated psychotic symptoms, impairments of social, emotional and cognitive functioning[2], and help-seeking behaviour[3]. They have around 20% risk of developing psychosis (but not any other non-psychotic disorder[4,5]) over a two-year period[6].

Primary indicated prevention in CHR-P individuals has the unique potential to alter the course of the disorder[7] and improve clinical outcomes[8]. Current international guidelines – such as those of the National Institute for Health and Care Excellence (NICE) and the European Psychiatric Association (EPA) – recommend that CHR-P individuals be primarily offered cognitive behavioural therapy (CBT) with or without family interventions[9,10]. However, while prophylactic treatment with antipsychotics is altogether prohibited by NICE guidelines[9], the EPA allows its use in the case of severe and progressive symptomatology[10].

The evidence supporting these partially conflicting recommendations is relatively unclear[11], despite several pairwise meta-analyses having been published to date[10,12-18]. For example, earlier meta-analyses concluded that no reliable recommendations with respect to specific interventions could be made, because studies were too heterogeneous[12], with comparable efficacy across different treatments[16] or no effects at all[17]. The most recent meta-analysis concluded that both CBT and antipsychotics are effective[13]. The other meta-analyses were affected by mistakes[19] or methodological limitations, such as the use of overall effect sizes computed across heterogeneous interventions of questionable clinical interpretability[10,12,18], inclusion of patients not assessed with standard CHR-P instruments (e.g., with schizotypal disorders[20])[12,13,15,18], inclusion of non-randomized and uncontrolled trials[10], pooling of time-dependent outcomes[21] in the same group (e.g., 6 and 12 months[18]) or no time stratification at all[13], or poor meta-analytical approaches[13]. Meta-analyses have acquired a major influence on clinical practice and guidelines[22], so they can be particularly harmful if they are of suboptimal quality.

Another problem is that the included trials involved a variety of specific interventions[12], which were inconsistently clustered in pairwise comparisons. For example, although CBT is

an umbrella term for a plethora of heterogeneous strategies[23], different CBT protocols have been lumped together, and the specific efficacy of each defining element or specific protocol remains unclear[24].

The objective of this network meta-analysis (NMA) was to summarize the available evidence about the specific efficacy of different preventive interventions in CHR-P individuals. NMA offers additional benefits over standard pairwise analyses in that the comparative efficacy of specific interventions can be estimated and ranked, even when two treatments have never been compared directly head-to-head[25]. Furthermore, since NMA can improve the precision of estimates by allowing integration of both direct and indirect treatment effect estimates[26], it is recommended over pairwise meta-analyses by the World Health Organization as a basis for clinical guidelines[27]. Therefore, NMA should be considered the highest level of evidence in CHR-P treatment guidelines[28].

METHODS

The protocol for this study was registered on PROSPERO (CRD42017069550). The study was conducted in accordance with the PRISMA statement[29].

Interventions included

We included all randomized controlled trials (RCTs) of pharmacological and/or non-pharmacological interventions for CHR-P individuals. We were *a priori* interested in the following non-pharmacological interventions: CBT (various protocols), psychoeducation, family therapy, supportive counselling, needs-based interventions (NBI), and integrated psychological therapies. We were also interested in the following pharmacological interventions: antipsychotics (olanzapine, risperidone, ziprasidone, aripiprazole) and novel/experimental pharmacotherapies (omega-3 fatty acids and D-serine). As indicated in the protocol, additional interventions emerging from the literature search were also considered (e.g., glycine and cognitive remediation).

The definition of the exact types of interventions is essential to reduce heterogeneity and produce robust informative results of direct clinical significance. As such, we first took each trial and carefully identified the treatment components that were characterizing each specific intervention, as detailed below.

Needs-based interventions (NBI)

Since CHR-P patients recruited in clinical trials are help-seeking adolescents and young adults accessing clinical services, randomizing them to no treatment is not considered a reasonable or ethical option[30]. Defining "treatment as usual" in these samples is also challenging, because treatment is not standardized and largely depends on local service configurations and the availability of specific resources or competences.

We therefore used the most established and original definition of NBI employed by the founders of the CHR-P paradigm, which focuses on the presenting symptoms and problems already manifest[31]. In accordance with this definition[32], NBI may include any of the following components: a) supportive psychotherapy primarily focusing on pertinent issues such as social relationships and vocational or family problems; b) case management, providing psychosocial assistance with accommodation, education or employment; c) brief family psychoeducation and support; d) medications other than antipsychotics; and e) clinical monitoring and crisis management [31,33].

Cognitive behavioural therapy, French & Morrison protocol (CBT-F)

The CBT-F protocol[34] is based on the principles developed by Beck[35]. The intervention is formulation-driven, problem-focused and time-limited, with manualized strategies selected on the basis of the patient's prioritized problem. The key components include building engagement, collaborative goal-setting and formulation, normalizing experiences, evaluating appraisals and core beliefs, and behavioural experiments[34,36].

Cognitive behavioural therapy, van der Gaag protocol (CBT-V)

The protocol developed by van der Gaag et al[37] essentially includes the French & Morrison protocol[34], but with two additional components. These comprise psychoeducation about dopamine system supersensitivity and training/behavioural experiments on cognitive biases that may contribute to paranoia[38]. Further behavioural goals include sustaining school and work attendance, enhancing social relationships, and reducing cannabis use[37].

Integrated psychological interventions, Bechdolf protocol (IPI)

The protocol developed by Bechdolf et al[39] contains a number of components, including individual CBT-F[34], manualized group social skills training, computerized cognitive remediation to address thought and perception deficits, and manualized psychoeducational multi-family group sessions[39,40].

Family-focused therapy, Miklowitz protocol (FFT)

A family-focused therapy (FFT) protocol, initially designed for those with or at risk of bipolar disorder, was adapted by Miklowitz et al[41] for the CHR-P population. The key components include psychoeducation and development of a prevention plan with the patient and family, sessions where the patient and family practice skills for better com-

munication, and sessions focusing on enhancing problem solving skills[41].

Pharmacological interventions

Pharmacological interventions included currently licensed medications, novel or experimental pharmacotherapies, and nutritional supplements.

Placebo

The placebo designation was reserved for placebo pills administered as pharmacological control conditions. Placebos were designed to match the active drug intervention in appearance but without the pharmacological compound of interest.

Nodes for the network meta-analysis

The specific interventions listed above were pooled into "nodes" for the network meta-analysis. Nodes were defined by the linear combination of any of the above specific interventions. Each individual pharmacological treatment was assigned to its own node. As indicated in the protocol, different dosages of the same drug/molecule were classed under the same node. Placebo was initially considered as a separate node from NBI. However, in line with the protocol, sensitivity analyses investigated the effect of alternate clustering of nodes (see statistical analysis).

Search strategy and selection criteria

We performed a multi-step literature search using the following keywords: (risk OR prodromal OR prodrom* OR ultra high risk OR clinical high risk OR high risk OR genetic high risk OR at risk mental state OR risk of progression OR progression to first-episode OR prodromally symptomatic OR basic symptoms) AND (psychosis) AND (RCT OR randomized controlled trial OR placebo controlled trial OR trial).

First, systematic searches were conducted in the Web of Science (which includes Web of Science Core Collection, BIOSIS Citation Index, KCI - Korean Journal Database, MEDLINE, Russian Science Citation Index, and SciELO Citation Index), the Cochrane Central Register of Controlled Trials, and Ovid/PsychINFO databases, until July 18, 2017, with no restrictions on language or publication date.

Second, we used Scopus/Web of Science to search reference lists of retrieved articles and previously conducted systematic reviews and meta-analyses. We manually searched for published and unpublished data in relevant conference proceedings, trial registries and drug-approval agencies. In addition, we contacted study authors for supplemental data and searched the OpenGrey database for grey literature.

Abstracts identified by this process were then screened, and full-text articles were retrieved for further inspection against the inclusion and exclusion criteria (as detailed *a priori* in the protocol). The literature search, study selection and data extraction were conducted by two authors (CD, UP) independently. During all stages, in the case of disagreement, consensus was reached through discussion with a third author (PFP).

Studies were eligible for inclusion when the following criteria were fulfilled: a) original articles, abstracts or pilot studies; b) RCTs (including cluster randomized trials, but excluding cross-over studies); c) designed as blinded (either single- or double-blind); d) conducted in CHR-P individuals as established by validated assessments, i.e. Comprehensive Assessment of At-Risk Mental States (CAARMS)[42], Structured Interview for Psychosis-risk Syndromes (SIPS)[43,44], Positive and Negative Syndrome Scale (PANSS)[45], Brief Psychiatric Rating Scale (BPRS)[46], or Early Recognition Inventory (ERIraos)[47]; e) comparing specific preventive interventions as defined above; and f) sample size of 10 or greater[48].

The exclusion criteria were: a) reviews/non-original data; b) studies lacking at least two compared groups; c) studies of first-episode psychosis or other non-CHR-P groups; d) lack of data needed for meta-analytical computation of the primary (transition) outcome (authors were contacted and asked to provide summary data); e) lack of proper randomization (quasi-randomization, observational naturalistic studies); f) samples size < 10; and g) articles presenting overlapping, redundant data (for a particular outcome at the same time point). Specifically, in the case of overlapping samples, we used the largest one. Studies that were designed as blinded but could not maintain blinding during follow-up (e.g., for psychological interventions) were not excluded.

Outcome measures and data extraction

The primary outcome was transition to psychosis. Due to the variable effect of time on transition risk[6,21], we stratified outcomes and analyses into 6 and 12 month follow-up time points. Sample sizes were based on the numbers randomized to each arm, to prevent artificial inflation of transition risk[6,49]. Participants who dropped out of individual studies after randomization were classified as non-transitions[6,10,14,50].

Where studies did not report sufficient data to extract the primary outcome, we contacted the relevant authors. In the case of non-response or where studies presented data graphically, numerical data were digitally extracted from the Kaplan-Meier plots using a previously validated procedure[51,52], as defined in the protocol.

The secondary outcome was the acceptability of interventions (discontinuation due to any cause), indexed as the number of participants who dropped out of each arm for any reason following randomization, over the number randomized [53-55].

In addition, we extracted the following information for each study: first author and year of publication, country, types of outcomes, intervention and control descriptions, study design,

quality assessment (see below), intervention period and follow-up duration, study arm details (sample size, mean age, percent male), and diagnostic tools used for CHR-P diagnosis and determining transition to psychosis.

Quality of the evidence

Risk of bias

The Cochrane Risk of Bias tool[56] was used to assess and classify the risk of bias in each of the included studies, as per criteria defined *a priori*. A judgement was made about whether each study had a high, low or unclear risk of bias in each of the following six domains: random sequence generation, allocation concealment, blinding of participants and study personnel, blinding of outcome assessments, incomplete outcome data, and selective outcome reporting.

The overall risk of bias was classified as low if none of the above domains was rated as high risk and three or less were rated as unclear risk. It was classified as moderate if one domain was rated as high risk, or none rated as high risk but four or more rated as unclear risk. All other studies were classified as having a high risk of bias[57].

To represent the quality of evidence associated with comparisons in the network meta-analysis, we used coloured edges in the network plots, as recommended[58].

GRADE

We assessed the certainty of evidence contributing to network estimates of the primary outcome using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework[59]. The GRADE method characterizes the quality of a body of evidence on the basis of six factors: study limitations, imprecision, heterogeneity, inconsistency, indirectness, and publication bias[59].

We tabulated the findings for the above six factors to aid in the decision-making process for the downgrading of evidence. If one of the factors was present for a comparison, then the overall confidence rating for that comparison was considered for downgrading by one or two levels (as appropriate). Each comparison started as high quality/confidence (as based on RCTs), and was downgraded to moderate, low or very low, depending on the presence, severity and potential impact of the aforementioned factors. These represented the final judgements about the certainty of the evidence[59,60].

Statistical analysis

Frequentist NMAs were conducted for transition and acceptability outcomes using the *network* package in STATA (version SE 14.2; StataCorp). First, a network plot was constructed for each outcome[61] to ensure that nodes of the network were sufficiently connected[58]. We then performed a NMA assuming consistency and a common heterogeneity across all comparisons in the network. This allowed us to derive a single summary treatment effect (odds ratio, OR) for every possible pairwise comparison of treatments, which takes account of all evidence from the network of trials, including both direct and indirect comparisons. Correlations in effect sizes induced by multi-arm trials[62] were accounted for[58,63]. The resulting relative ORs with 95% confidence intervals (CIs) for each pair of treatments were reported in league tables[64].

The interventions were then ranked by the surface under the cumulative ranking curve (SUCRA), which accounts for the location as well as the variance of all relative treatment effects[65]. SUCRA is a numeric presentation of the overall ranking and provides a single number (from 0 to 100%) associated with each intervention[66]. The higher the SUCRA value, and the closer to 100%, the higher the likelihood that an intervention is in the top rank, and vice versa[66]. Cluster ranking methods[58,65] – using both transition and acceptability SUCRA values – were used to order the treatments in league tables, in line with recent guidance which requires interpretation of SUCRA only in the context of NMA uncertainty, rather than at face value[66]. Statistical significance was set at $p < 0.05$.

We assessed the assumption of consistency by calculating, for each closed loop, an inconsistency factor (differences between direct and indirect evidence) along with 95% CIs and associated p values. We plotted the results graphically as the ratio of ORs (RORs) and 95% CIs for each loop[64]. Inconsistency was defined as disagreement between direct and indirect evidence, with 95% CIs for RORs excluding 1.

Given the low power of the loop-specific approach and its focus on local inconsistency (between direct and indirect evidence), we also tested a full design-by-treatment model[62] for the primary outcome to evaluate inconsistency more globally, including between trials with different designs (e.g., two-arm vs. multi-arm). A NMA under the inconsistency model was applied and a χ^2 test was used to infer about the statistical significance of all possible inconsistencies in the networks[67].

The transitivity assumption was examined by assessing the distributions of potential effect modifiers for every comparison in the network, including percentage of males[68], age[69], percentage exposed to antipsychotic medications at baseline[70], type of blinding and publication year[6]. The presence of small-study effects was assessed by visual inspection of comparison-adjusted funnel plots[59].

To evaluate the impact of study quality and our data analysis procedures, we conducted sensitivity analyses for the primary outcome restricted to: a) studies with a low risk of bias for the blinding of outcome assessments; b) studies whose data were not digitally extracted (e.g., from Kaplan-Meier plots); and c) published data only. We also repeated the analyses after applying alternate clustering of the following nodes: a) pooling NBI and placebo; b) pooling different CBT protocols; c) pooling different types of antipsychotic molecules, and d) separating the different NBI components (i.e., supportive therapy vs. clinical monitoring vs. other). Finally, network meta-regressions

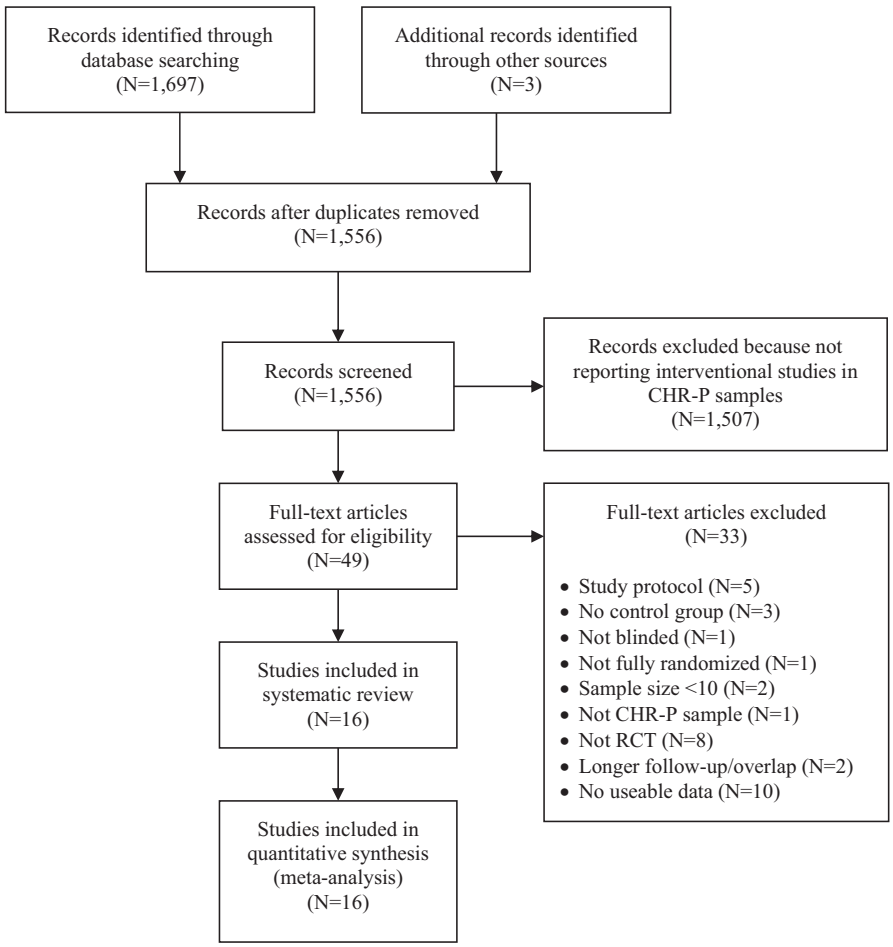

Figure 1 PRISMA flow chart of the study selection process. CHR-P – clinical high risk for psychosis, RCT – randomized controlled trial

were planned in the case of substantial heterogeneity and at least ten studies[71] to test the impact of different CHR-P diagnostic instruments/criteria.

RESULTS

Characteristics of the included studies

1,556 references were found in the literature search, most of which were not reporting RCTs in CHR-P individuals; 49 were fully screened for the inclusion and exclusion criteria, resulting in a final sample of 16 studies (Figure 1). There were only five, four, two and three studies reporting data for 18, 24, 36 and >36 month time points, respectively, and therefore all results reported hereafter are for 6 and 12 months only.

The 16 studies used in the analyses of the primary outcome contributed data on 2,035 patients, with a mean age of 20.1 ± 2.8 years, and 57% were male (Table 1). The mean sample size was 127 (range 44-304). Six studies were conducted in North America, six in Europe, three in Australia and one was multinational. Two studies were three-arm and the rest were two-arm trials. Two studies had a treatment duration of <6 months, ten of 6 months, and four of 12 months. Of the 14 studies with available information on sponsorship/funding, three[31,75,81,82] acknowledged pharmaceutical company grants. The CAARMS and the SIPS were the most common CHR-P diagnostic instruments[44] (six and seven studies, respectively).

For the 6-month analysis of the primary outcome, these 16 studies provided data on 20 direct comparisons between 11 different treatment nodes (Figure 2). Three studies provided follow-up data only for the 6-month analysis, and therefore the 12-month analysis consisted of 13 studies (N=1,811), providing data on 17 direct comparisons between 8 different treatment nodes (Figure 2). The network plots for the acceptability outcome were the same at 12 months and similar at 6 months (integrated psychological interventions was missing).

Primary outcome: transition

Results of the NMA showed a lack of evidence for clearly superior efficacy of specific treatments in preventing transi-

Table 1 Details of included studies

Study	Study arms (N)	Network inclusion	Treatment duration (months)	Follow-up time points (months)	% male	Mean age	CHR-P criteria	Study design	Country	% exposed to antipsychotics at baseline
Addington et al[30]	CBT-F + NBI (27)	6, 12	6	6,12,18	71	20.9	SIPS	SB-RCT	Canada	0
	NBI (24)									
Amminger et al[33]	Omega-3 + NBI (41)	6, 12	3	6,12,84	33	16.4	PANSS	DB-RCT	Austria	0
	NBI (40)									
Bechdolf et al[39]	IPI (63)	6, 12	12	6,12,18, 24	63	26.0	ERIraos	SB-RCT	Germany	0
	NBI (65)									
Bechdolf et al[72]	NBI + ARI (96)	6, 12	12	6,12	66	24.4	SIPS + BS	SB-RCT	Germany	3.4
	NBI (55)									
	CBT-F + NBI (129)									
Cadenhead et al[73]	Omega-3 + NBI (65)	6, 12	6	6,12,18, 24	56	18.8	SIPS	DB-RCT	US, Canada	0
	NBI (62)									
Kantrowitz et al[74]	D-serine + NBI (20)	6	4	4	66	19.4	SIPS	DB-RCT	US	11.4
	NBI (24)									
McGlashan et al[75]	NBI + OLA (31)	6, 12	12	12, 24	65	17.7	SIPS	DB-RCT	US, Canada	10
	NBI (29)									
McGorry et al[31]	CBT-F + RIS + NBI (31)	6, 12	6	6,12,36-48	58	20.0	BPRS	SB-RCT	Australia	0
	NBI (28)									
McGorry et al[76]	Omega-3 + NBI (153)	6, 12	6	6,12	46	19.2	CAARMS	DB-RCT	Multi-national	0
	NBI (151)									
Miklowitz et al[41]	FFT + NBI (66)	6	6	6	57	17.4	SIPS	SB-RCT	US, Canada	20.9
	NBI (63)									

Table 1 Details of included studies *(continued)*

Study	Study arms (N)	Network inclusion	Treatment duration (months)	Follow-up time points (months)	% male	Mean age	CHR-P criteria	Study design	Country	% exposed to antipsychotics at baseline
Morrison et al[36]	CBT-F + NBI (37)	6, 12	6	6,12,36	69	22.0	CAARMS	SB-RCT	UK	0
	NBI (23)									
Morrison et al[77]	CBT-F + NBI (144)	6, 12	6	6,12,18,24	63	20.7	CAARMS	SB-RCT	UK	0
	NBI (144)									
Stain et al[78]	CBT-F + NBI (30)	6, 12	6	6,12	40	16.3	CAARMS	SB-RCT	Australia	0
	NBI (27)									
van der Gaag et al[37]	CBT-F + CBT-V + NBI (98)	6, 12	6	6,12,18,48	49	22.7	CAARMS	SB-RCT	The Netherlands	1.5
	CBT-F + NBI (103)									
Woods et al[79] Woods[80]	NBI + ZIP (24)	6	6	6	64	22.3	SIPS	DB-RCT	US	0
	NBI (27)									
Yung et al[81] McGorry et al[82]	CBT-F + NBI (44)	6, 12	12	6,12	39	18.1	CAARMS	SB-RCT	Australia	0
	CBT-F + RIS + NBI (43)									
	NBI (28)									

CBT – cognitive behavioural therapy, CBT-F – French & Morrison CBT protocol, NBI – needs-based interventions (including placebo), IPI – integrated psychological interventions, ARI – aripiprazole, OLA – olanzapine, RIS – risperidone, FFT – family-focused therapy, CBT-V – van der Gaag CBT protocol, ZIP – ziprasidone, SIPS – Structured Interview for Psychosis-risk Syndromes, PANSS – Positive and Negative Syndrome Scale, ERIraos – Early Recognition Inventory, BS – basic symptoms, BPRS – Brief Psychiatric Rating Scale, CAARMS – Comprehensive Assessment of At-Risk Mental States, SB-RCT – single-blind randomized controlled trial, DB-RCT – double-blind randomized controlled trial

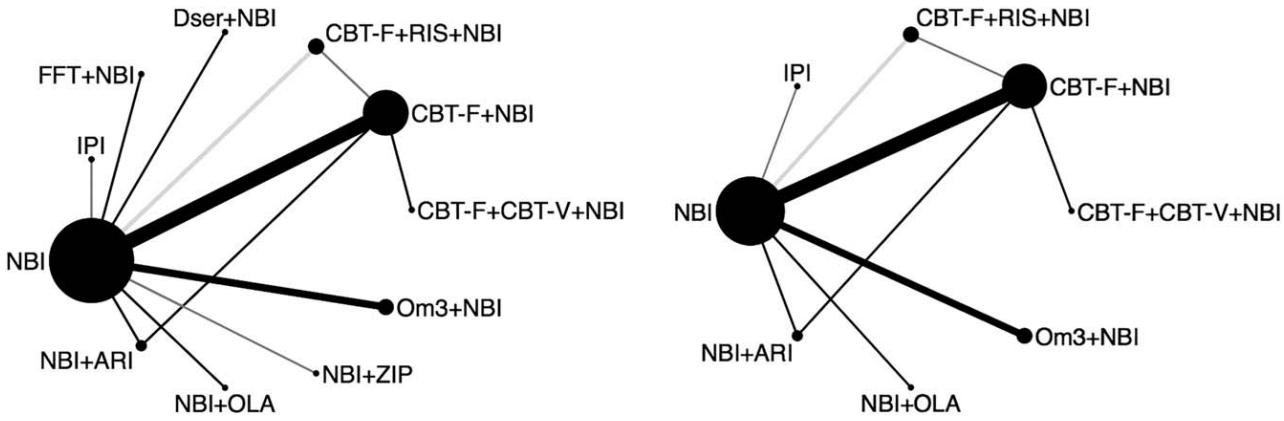

Figure 2 Network plots of direct comparisons in the network meta-analysis for transition outcome at 6 (on the left) and 12 months (on the right). The width of the lines is proportional to the number of trials comparing each pair of treatments and the size of each node is proportional to the number of studies testing the specific treatment. The color of the lines represents the comparison-specific bias level for the blinding of outcome assessments in the majority of trials (black = low risk, dark grey = unclear risk, light grey = high risk). NBI – needs-based interventions (including placebo), IPI – integrated psychological interventions, FFT – family-focused therapy, Dser – D-serine, CBT – cognitive behavioural therapy, CBT-F – French & Morrison CBT protocol, CBT-V – van der Gaag CBT protocol, RIS – risperidone, Om3 – omega-3 fatty acids, ZIP – ziprasidone, OLA – olanzapine, ARI – aripiprazole.

tion, with no significant effects of any one intervention over any others at 6 or 12 month time points (Figures 3 and 4).

Using NBI as a comparator, the OR and 95% CI for each treatment (all OR < 1 favor the given treatment) at 6 months were: 0.06 (0.00-1.90) for integrated psychological interventions; 0.17 (0.01-2.69) for family-focused therapy + NBI; 0.22 (0.02-2.17) for CBT-F + CBT-V + NBI; 0.29 (0.03-2.57) for olanzapine + NBI; 0.21 (0.04-1.08) for CBT-F + risperidone + NBI; 0.52 (0.03-10.72) for ziprasidone + NBI; 0.56 (0.03-11.51) for D-serine + NBI; 0.64 (0.15-2.68) for omega-3 + NBI; 0.73 (0.27-2.01) for CBT-F + NBI; and 0.94 (0.15-5.73) for aripiprazole + NBI.

At 12 months, ORs against the NBI comparator were: 0.04 (0.00-1.06) for integrated psychological interventions; 0.15 (0.02-1.25) for olanzapine + NBI; 0.21 (0.03-1.60) for CBT-F + CBT-V + NBI; 0.43 (0.11-1.68) for CBT-F + risperidone + NBI; 0.58 (0.23-1.47) for CBT-F + NBI; 0.64 (0.18-2.26) for omega-3 + NBI; and 1.39 (0.26-7.28) for aripiprazole + NBI.

While almost all the interventions at both time points had estimates favoring them over NBI, the differences were not beyond chance, and the 95% CIs for the NMA estimates were often very large, indicating substantial imprecision. The cluster ranking (based on SUCRA values for transition and acceptability) at 6 and 12 months is illustrated by the ordering of treatments in Tables 2 and 3.

No statistically significant inconsistency was evident at any time point, with 95% CIs for all RORs compatible with zero inconsistency (ROR=1). However, only two loops were available. Using the design-by-treatment interaction test[62], we found no evidence for significant inconsistency for 6 month (p=0.90) and 12 month (p=0.93) networks.

Only two studies had an overall low risk of bias[33,79]; five had unclear risk[72-76], and nine had high risk[30,31,36,37,39,41,77,78,81]. The edges (lines) in Figure 2 reflect the Cochrane risk of bias for the blinding of outcome assessments, estimated as the level of bias in the majority of trials and weighted according to the number of studies in each comparison[58]. The GRADE assessment highlighted low or very low confidence in almost all estimates, primarily due to study limitations (high risks of bias) and imprecision.

The numbers of studies remaining (at 6 and 12 months, respectively) after exclusion of those with a high or unclear risk of bias for the blinding of outcome assessments were 10 and 8; after exclusion of those whose data were extracted by digitizing Kaplan-Meier plots were 13 and 12; after exclusion of unpublished studies were 13 and 11. The NMA model was refitted accordingly and no differences in conclusions were observed for any OR at any time point.

Repeating the analyses treating NBI + placebo as a separate node to NBI, or separating the different NBI components, had no effect on the NMA estimates, and therefore we used the pooled NBI + placebo in the main analysis (Table 1, Figures 2-4). Similarly, pooling together different CBT protocols or different antipsychotic molecules in the same node produced no significant results. There were not enough studies to allow robust meta-regression analyses on the type of CHR-P instruments. Visual inspection of funnel plots revealed no substantive evidence of small-study effects.

Secondary outcome: acceptability

Acceptability data were available for 14 of 16 studies at 6 months (N=1,848), and 12 of 13 studies at 12 months (N=1,752). There were no significant differences in acceptability between any treatment comparisons at 6 or 12 months (Figures 3 and 4). The SUCRA cluster ranking (for transition and acceptability) is illustrated in those figures.

IPI	-	-	-	-	-	-	-	-	-	-
0.39 (0.00 to 31.26)	FFT + NBI	0.58 (0.12 to 2.73)	0.20 (0.03 to 1.18)	0.86 (0.18 to 4.15)	0.38 (0.07 to 2.01)	0.69 (0.13 to 3.85)	0.59 (0.17 to 2.03)	0.67 (0.21 to 2.14)	0.84 (0.21 to 3.38)	0.59 (0.20 to 1.70)
0.29 (0.00 to 17.13)	0.74 (0.02 to 27.15)	CBT-F + CBT-V +NBI	0.34 (0.05 to 2.09)	1.48 (0.32 to 6.85)	0.65 (0.12 to 3.56)	1.19 (0.21 to 6.82)	1.01 (0.28 to 3.65)	1.15 (0.41 to 3.21)	1.43 (0.37 to 5.60)	1.01 (0.33 to 3.08)
0.22 (0.00 to 12.43)	0.57 (0.02 to 19.56)	0.77 (0.03 to 17.86)	OLA + NBI	4.37 (0.69 to 27.70)	1.92 (0.28 to 13.20)	3.52 (0.49 to 25.15)	2.97 (0.62 to 14.36)	3.41 (0.76 to 15.42)	4.23 (0.77 to 23.21)	2.98 (0.71 to 12.54)
0.31 (0.01 to 13.33)	0.79 (0.03 to 20.17)	1.07 (0.07 to 15.83)	1.40 (0.09 to 21.37)	CBT-F + RIS + NBI	0.44 (0.08 to 2.48)	0.81 (0.14 to 4.74)	0.68 (0.18 to 2.56)	0.78 (0.25 to 2.45)	0.97 (0.23 to 4.05)	0.68 (0.21 to 2.17)
0.12 (0.00 to 11.54)	0.32 (0.01 to 19.30)	0.43 (0.01 to 18.69)	0.56 (0.01 to 23.08)	0.40 (0.01 to 12.41)	ZIP + NBI	1.83 (0.29 to 11.69)	1.55 (0.37 to 6.48)	1.77 (0.46 to 6.91)	2.20 (0.46 to 10.59)	1.55 (0.43 to 5.57)
0.12 (0.00 to 10.94)	0.30 (0.00 to 18.30)	0.40 (0.01 to 17.74)	0.52 (0.01 to 21.90)	0.38 (0.01 to 11.79)	0.94 (0.01 to 67.94)	D-serine + NBI	0.85 (0.19 to 3.74)	0.97 (0.24 to 4.00)	1.20 (0.24 to 6.09)	0.85 (0.22 to 3.24)
0.10 (0.00 to 3.98)	0.26 (0.01 to 5.94)	0.35 (0.02 to 5.11)	0.45 (0.03 to 6.17)	0.33 (0.04 to 2.93)	0.82 (0.03 to 23.12)	0.87 (0.03 to 24.80)	Om3 + NBI	1.15 (0.52 to 2.51)	1.42 (0.47 to 4.33)	1.00 (0.53 to 1.90)
0.09 (0.00 to 3.02)	0.23 (0.01 to 4.38)	0.30 (0.04 to 2.34)	0.40 (0.04 to 4.39)	0.28 (0.05 to 1.66)	0.71 (0.03 to 17.27)	0.76 (0.03 to 18.53)	0.88 (0.15 to 5.04)	CBT-F + NBI	1.24 (0.50 to 3.06)	0.87 (0.55 to 1.37)
0.07 (0.00 to 3.19)	0.18 (0.01 to 4.89)	0.24 (0.02 to 3.56)	0.31 (0.02 to 5.26)	0.22 (0.02 to 2.38)	0.56 (0.02 to 18.82)	0.59 (0.02 to 20.18)	0.68 (0.07 to 6.84)	0.78 (0.13 to 4.64)	ARI + NBI	0.70 (0.28 to 1.74)
0.06 (0.00 to 1.90)	0.17 (0.01 to 2.69)	0.22 (0.02 to 2.17)	0.29 (0.03 to 2.57)	0.21 (0.04 to 1.08)	0.52 (0.03 to 10.72)	0.56 (0.03 to 11.51)	0.64 (0.15 to 2.68)	0.73 (0.27 to 2.01)	0.94 (0.15 to 5.73)	NBI

Comparison Transition Acceptability

Figure 3 Relative effect sizes for transition to psychosis and acceptability (dropout for any reason) at 6 months, odds ratios (95% CI). Comparisons between treatments should be read from left to right, and the estimate is in the cell in common between the column-defining treatment and the row-defining treatment. Treatments are reported in descending order (from top left to bottom right) as per the cluster ranking for transition and acceptability. For transition, an OR less than 1 favors the column-defined treatment. For acceptability, an OR less than 1 favors the row-defined treatment. All 95% CIs include the null hypothesis OR = 1. Dashes (-) indicate no available NMA estimate. CBT – cognitive behavioural therapy, CBT-F – French & Morrison CBT protocol, CBT-V – van der Gaag CBT protocol, NBI – needs-based interventions (including placebo), RIS – risperidone, FFT – family-focused therapy, IPI – integrated psychological interventions, ARI – aripiprazole, OLA – olanzapine, ZIP – ziprasidone, Om3 – omega-3 fatty acids.

[38]

IPI	0.73 (0.15 to 3.48)	1.49 (0.34 to 6.54)	1.43 (0.35 to 5.89)	1.72 (0.55 to 5.43)	1.37 (0.41 to 4.54)	1.68 (0.57 to 4.91)	2.72 (0.75 to 9.83)
0.26 (0.01 to 12.94)	**OLA + NBI**	2.05 (0.45 to 9.45)	1.97 (0.46 to 8.52)	2.37 (0.71 to 7.94)	1.89 (0.54 to 6.63)	2.31 (0.74 to 7.20)	3.74 (0.98 to 14.29)
0.19 (0.00 to 9.17)	0.73 (0.04 to 13.83)	**CBT-F + CBT-V +NBI**	0.96 (0.26 to 3.51)	1.15 (0.45 to 2.93)	0.92 (0.30 to 2.86)	1.12 (0.41 to 3.11)	1.82 (0.57 to 5.84)
0.09 (0.00 to 3.23)	0.35 (0.03 to 4.35)	0.48 (0.05 to 5.03)	**CBT-F + RIS + NBI**	1.20 (0.49 to 2.95)	0.96 (0.33 to 2.74)	1.17 (0.47 to 2.93)	1.89 (0.62 to 5.76)
0.07 (0.00 to 2.06)	0.26 (0.03 to 2.60)	0.35 (0.06 to 2.20)	0.74 (0.17 to 3.22)	**CBT-F + NBI**	0.80 (0.42 to 1.52)	0.97 (0.65 to 1.47)	1.58 (0.78 to 3.18)
0.06 (0.00 to 2.07)	0.24 (0.02 to 2.74)	0.32 (0.03 to 3.52)	0.67 (0.10 to 4.24)	0.90 (0.19 to 4.28)	**Om3 + NBI**	1.22 (0.72 to 2.07)	1.98 (0.82 to 4.80)
0.04 (0.00 to 1.06)	0.15 (0.02 to 1.25)	0.21 (0.03 to 1.60)	0.43 (0.11 to 1.68)	0.58 (0.23 to 1.47)	0.64 (0.18 to 2.26)	**NBI**	1.62 (0.80 to 3.29)
0.03 (0.00 to 1.13)	0.11 (0.01 to 1.60)	0.15 (0.01 to 1.72)	0.31 (0.04 to 2.47)	0.42 (0.08 to 2.14)	0.46 (0.06 to 3.72)	0.72 (0.14 to 3.78)	**ARI + NBI**

Comparison Transition Acceptability

Figure 4 Relative effect sizes for transition to psychosis and acceptability (dropout for any reason) at 12 months, odds ratios (95% CI). Comparisons between treatments should be read from left to right, and the estimate is in the cell in common between the column-defining treatment and the row-defining treatment. Treatments are reported in descending order (from top left to bottom right) as per the cluster ranking for transition and acceptability. For transition, an OR less than 1 favors the column-defined treatment. For acceptability, an OR less than 1 favors the row-defined treatment. All 95% CIs include the null hypothesis OR = 1. CBT – cognitive behavioural therapy, CBT-F – French & Morrison CBT protocol, CBT-V – van der Gaag CBT protocol, NBI – needs-based interventions (including placebo), RIS – risperidone, IPI – integrated psychological interventions, ARI – aripiprazole, OLA – olanzapine, Om3 – omega-3 fatty acids.

DISCUSSION

This is the first network meta-analysis exploring the efficacy of specific interventions for the prevention of psychosis in CHR-P individuals. Adopting strict inclusion criteria, a total of 16 RCTs, with 2,035 patients, were included in the analyses. There were not enough studies to analyze data with a NMA approach beyond 6 and 12 month follow-ups. Two networks were established at 6 and 12 months, including 11 and 8 nodes, respectively. Network meta-analyses showed no clear evidence of superior efficacy for any specific intervention at any time point. The results were not affected by biases, inconsistency or small-study effects.

The main finding of the current study is that there is a lack of evidence to favor specific effective interventions to prevent psychosis in CHR-P individuals. Our analyses were based on a detailed protocol, which defined the exact type of interventions and nodes *a priori*. This was done with the aim of providing robust informative results of direct clinical significance. For example, deconstructing the efficacy of different types of CBT that are based on different protocols[83] seems necessary to inform accurate and evidence-based clinical guidelines for patients, clinicians and policy makers. Our NMA comparing the different CBT protocols is also timely, since authors have recently claimed that the "black box" of CBT should be unpacked into its specific therapeutic components[23,24,84-86].

In a similar fashion, our NMA represents the first attempt at deconstructing – through sensitivity analyses – the effect of different components (including placebo) that characterize NBI, which is usually employed as the control condition in this field. We also restricted our literature search to include only RCTs designed to be blinded, and studies that strictly used CHR-P assessment instruments, to minimize selection biases. Therefore, to date, our study represents the most fine-grained analysis that has deconstructed the specific effect of preventive interventions for psychosis.

Negative (non-significant) results are rarely published in psychiatric literature[87], which is affected by excess of statistical significance[88-92]. In fact, interpreting negative findings is particularly challenging, because absence of evidence is not evidence of absence[93]. In particular, when large CIs are observed (as in Figures 3 and 4), some sizeable effects may still have been missed. Nevertheless, our work represents the most powered data synthesis in this field. For example, the meta-analysis by Stafford et al[15] – on which current clinical guidelines are based – analyzed 11 studies, but one of them included an open-label trial (N=124)[94] and another did not assess participants against standard CHR-P criteria (N=79)[20], leaving nine studies (N=1,043) that are in common with the current

NMA. Since that meta-analysis, seven new trials involving 992 new CHR-P participants (an increase of more than 50%) have been published, all of which reported non-significant effects[41,72-74,76,78-80]. Since our NMA included these new data, it is more powered than previous pairwise analyses.

In the context of power considerations, indirect evidence, when combined with direct evidence through NMA, increases the power and precision of treatment effect estimates compared to pairwise analyses[26]. Furthermore, when we pooled different CBT protocols or antipsychotic molecules in the same node – thus increasing the statistical power – no significant results were still observed. Overall, the core result of our NMA is more congruent with the evidence emerging from the most recent trials, compared to previous evidence syntheses.

The current lack of evidence to support specific preventive treatments is also consistent with the fact that the three largest interventional studies in this field have all produced negative findings[95]. Earlier studies that dominated the conclusions of some previous meta-analyses (e.g., the omega-3 trial[33]) were likely false positives. There is also converging lack of significant benefits on other clinical outcomes besides transition to psychosis, such as attenuated symptom severity[14,15,96], functioning[10,14,18], depressive comorbidities[15], distress[14], and quality of life[14,15].

These findings, taken together, are particularly problematic given the conceptual concerns over the clinical validity and significance of the dichotomous concept of transition within the CHR-P paradigm[97,98]. More to the point, it is not clear whether the currently tested treatments are only delaying the onset of psychosis as opposed to altering the course of the disorder[7]. Long-term outcome trials are scarce and the results are conflicting.

The additional caveat is that the exact mechanism of action of the tested preventive treatments is – at best – poorly defined, due to lack of an established and validated pathophysiological model underlying the onset of psychosis in CHR-P samples. A lack of mechanistic models forces researchers to proceed with empirical attempts that may eventually prove unsuccessful, as has ultimately been the case for omega-3 fatty acids[76]. However, as our ability to stratify CHR-P individuals into more homogenous subtypes improves, so may our success in testing specific treatments targeted to underlying biological and psychological mechanisms[99].

Our findings may have an impact on research and clinical practice. In times of scarce resources, our NMA can help to focus the next generation of research on the most promising interventions. Although our ranking analysis should be interpreted cautiously[66,100] in the context of non-superiority of any intervention compared to any other, it suggests that CBT-F, which currently represents the most widely adopted intervention, may not be the best candidate (of relevance, the largest CBT-F trial to date provided non-significant results[77]). On the other hand, the apparent promising profile of integrated psychological approaches could be the target of future replications.

Future research in this area will need to test novel interventions that may act on underlying psychological or neurobiological processes associated with the onset of psychosis. Although there are no clinically valid CHR-P biomarkers yet available[101], several international consortia are ongoing (PRONIA[102], NAPLS[103], PSYSCAN[104]) with the aim of developing them. At the same time, it seems warranted to address the clinical heterogeneity[1,6,49,105,106] that may prevent the discovery of reliable preventive treatments, and to improve the design of the next generation of trials. For example, it is apparent that unstructured recruitment processes and risk enrichment procedures in samples undergoing CHR-P assessment have a substantial role in determining the actual level of risk for psychosis in these individuals[107-109], leading to underpowered and non-significant trials[95]. On a clinical side, individuals meeting CHR-P criteria may be informed that, at present, there is no evidence for specific treatments being more effective than any others, and current options should be carefully weighted on a personal basis depending on an individual's needs.

This study has some limitations. First, only 16 RCTs were included, reflecting the paucity of high-quality studies available in the CHR-P field. However, capitalizing on the increased power and precision of NMA[26], the Cochrane group has conducted such analysis in even smaller databases, including as few as three to seven studies[110-113]. Furthermore, sufficient data were available for 6 and 12 month networks only, which precluded insight into whether treatments may have some effectiveness in the longer term. As a result of the sparse literature, many nodes were not well connected, with the corollary of limited ability to check for inconsistency, more imprecise estimates and wide 95% CIs.

In addition, the quality of NMA rests on the quality of included studies, many of which were found to be at high or unclear risk of bias, with GRADE confidence estimates predominantly low or very low – suggesting that true effects may be substantially different from the estimates. This is particularly the case for trials including any psychological interventions. We addressed this issue through a strict and detailed assessment of biases and sensitivity analyses. Going forward, given that all comparisons in the NMA were downgraded due to study limitations (risk of bias) and imprecision, the addition of high-quality studies with adequate sample sizes is needed to improve these confidence ratings.

A final limitation is that, whilst dropout due to any cause was available from the majority of trials, this is a rather crude measure of treatment acceptability, and a more proximal index, such as specific adverse effects, may have revealed significant differences between treatments, in particular for trials of antipsychotic molecules. However, these outcomes are rarely reported in the CHR-P literature.

In conclusion, there is currently no evidence to favor specific interventions for the prevention of psychosis. Further experimental research in this field is needed.

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2.2. COCHRANE RISK OF BIAS

World Psychiatry does not allow supplementary material so for completeness, details of the risk of bias assessment are presented below.

Risk of bias of the included studies

Only 2 studies had an overall low risk of bias (Amminger *et al*, 2010; Woods *et al*, 2017), 5 had unclear risk (Bechdolf *et al*, 2016; Cadenhead *et al*, 2017; Kantrowitz *et al*, 2015; McGlashan *et al*, 2006; McGorry *et al*, 2017), and 9 had a high risk (Addington *et al*, 2011; Bechdolf *et al*, 2012; van der Gaag *et al*, 2012; McGorry *et al*, 2002; Miklowitz *et al*, 2014; Morrison *et al*, 2004, 2012; Stain *et al*, 2016; Yung *et al*, 2011) (**Figure 2–1** below). In terms of individual bias domains (**Figure 2–2**), as may be expected from RCTs, 12/16 (75%) of studies were rated as a low risk of bias for both random sequence generation and allocation concealment, with the remaining studies having an unclear risk in these domains. However, 13/16 (81%) of studies had a high risk of bias owing to incomplete outcome data, which has previously been noted as an issue in CHR-P research due to high attrition. An inherent issue with RCTs of psychological therapies is the inability to blind participants and therapists to the treatment received, which is reflected by 9/16 (56%) of the studies having a high risk of performance bias. However, 6 studies (38%) had a low risk of performance bias—these were exclusively studies of pharmacological treatments, where it is possible to implement a true placebo-controlled double-blind design. Nevertheless, the blinding of outcome assessments—arguably one of the key bias domains—compared more favourably, with approximately 11 (69%), 3 (19%), and 2 (13%) studies having a low, unclear, and high risk of bias, respectively.

Figure 2–1. Risk of bias summary: each risk of bias item per study.

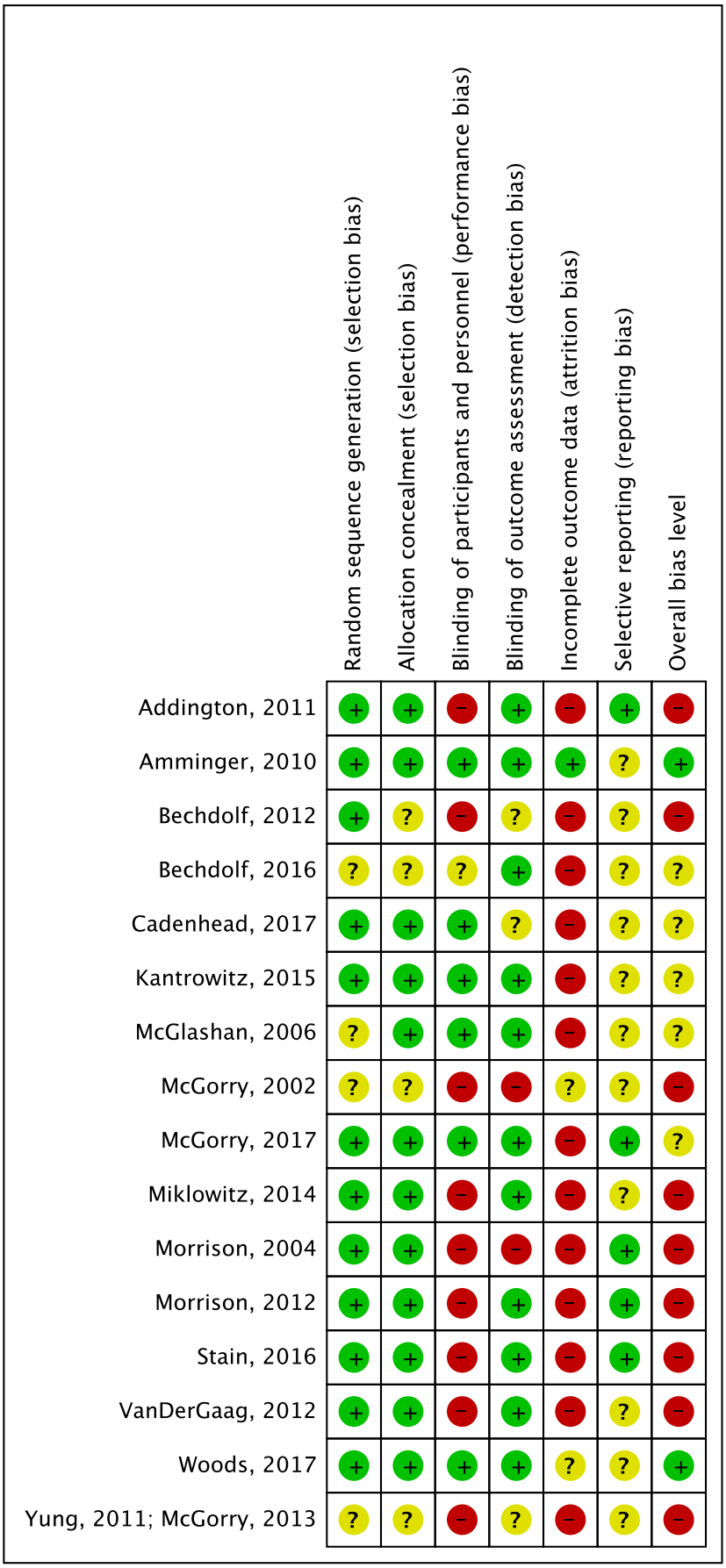
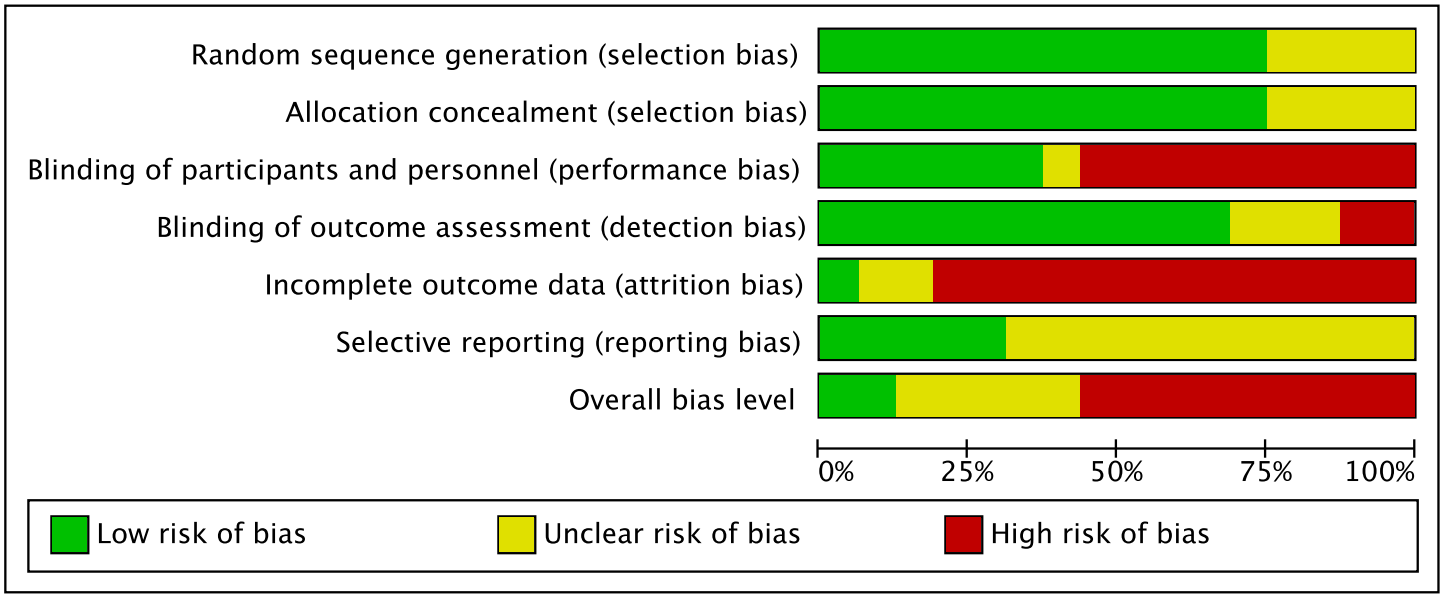


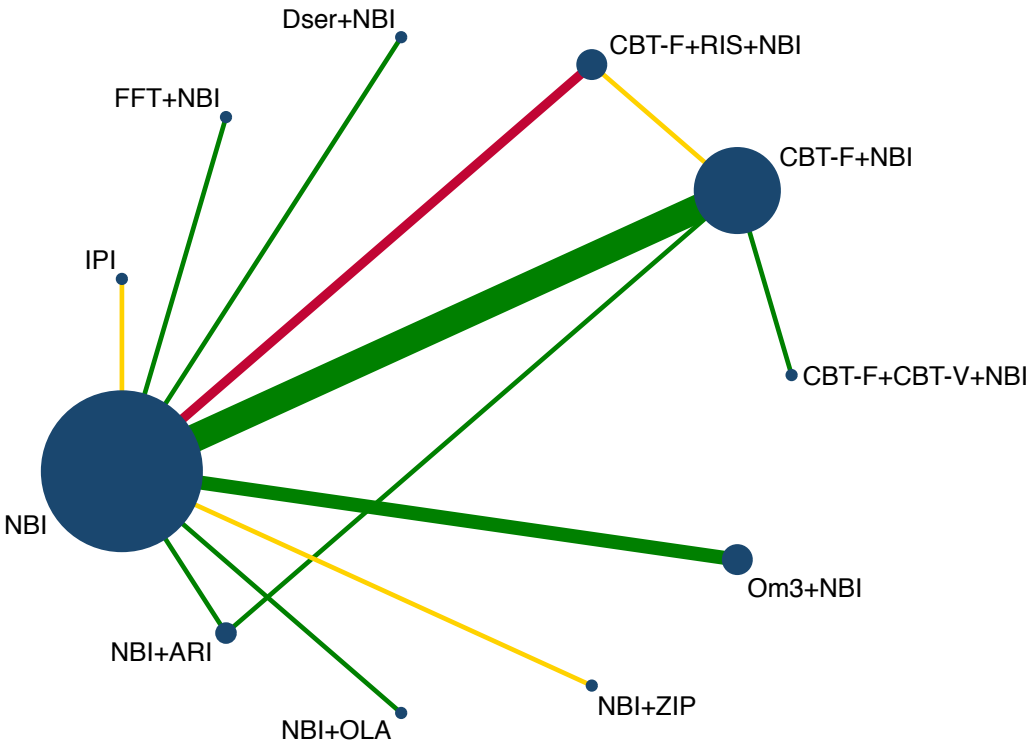
Figure 2–2. Risk of bias: each risk of bias item presented as percentages across all included studies.



Network plots of direct comparisons for the network meta-analysis (transition outcome) at 6 and 12 months, showing risk of bias for outcome assessment blinding.

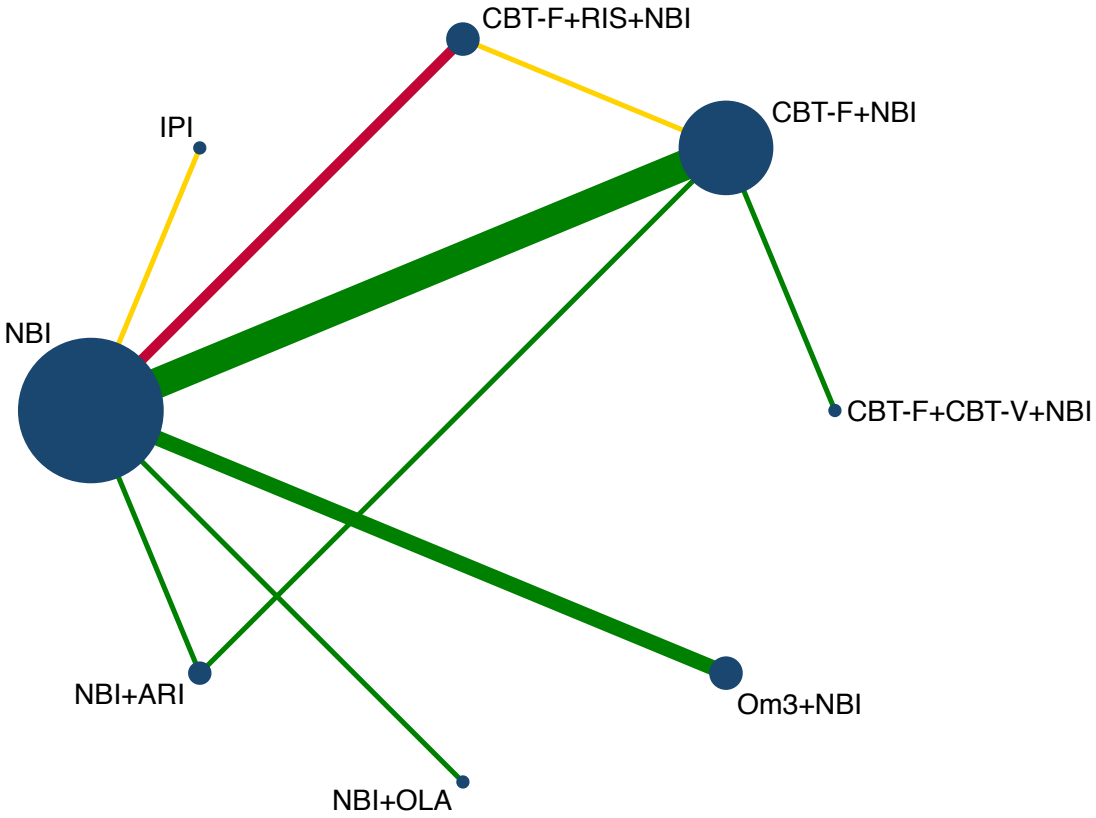
The width of the lines is proportional to the number of trials comparing each pair of treatments and the size of each node is proportional to the number of studies testing the specific treatment. The colour of the lines represents the comparison-specific bias level, estimated as the risk of bias level for the blinding of outcome assessments in the majority of included studies in each comparison (green = low risk, yellow= unclear risk, red= high risk).

Figure 2–3. Network plots of direct comparisons for the network meta-analysis for transition outcome at 6 months.



Treatment abbreviations (throughout this thesis). CBT-F = French & Morrison's CBT protocol (French and Morrison, 2004); CBT-V = van der Gaag et al's CBT protocol (van der Gaag *et al*, 2012); NBI = Needs-based interventions; RIS = risperidone; Dser = D-serine; FFT = Family-focused therapy (Miklowitz *et al*, 2014); IPI = Integrated Psychological Interventions (Bechdolf *et al*, 2012); ARI = aripiprazole; OLA= olanzapine; ZIP = ziprasidone; Om3 = omega-3 fatty-acids.

Figure 2–4. Network plots of direct comparisons for the network meta-analysis for transition outcome at 12 months.



3. NETWORK META-ANALYSIS – ATTENUATED PSYCHOTIC SYMPTOMS

3.1. PAPER 2 – NETWORK META-ANALYSIS – ATTENUATED PSYCHOTIC SYMPTOMS

Davies C, Radua J, Cipriani A, Stahl D, Provenzani U, McGuire P, et al (2018). Efficacy and acceptability of interventions for attenuated positive psychotic symptoms in individuals at clinical high risk of psychosis: a network meta-analysis. *Front Psychiatry* 9:187. https://doi.org/10.3389/fpsyt.2018.00187

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Efficacy and Acceptability of Interventions for Attenuated Positive Psychotic Symptoms in Individuals at Clinical High Risk of Psychosis: A Network Meta-Analysis

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Background: Attenuated positive psychotic symptoms represent the defining features of the clinical high-risk for psychosis (CHR-P) criteria. The effectiveness of each available treatment for reducing attenuated positive psychotic symptoms remains undetermined. This network meta-analysis (NMA) investigates the consistency and magnitude of the effects of treatments on attenuated positive psychotic symptoms in CHR-P individuals, weighting the findings for acceptability.

Methods: Web of Science (MEDLINE), PsycInfo, CENTRAL and unpublished/gray literature were searched up to July 18, 2017. Randomized controlled trials in CHR-P individuals, comparing at least two interventions and reporting on attenuated positive psychotic symptoms at follow-up were included, following PRISMA guidelines. The primary outcome (efficacy) was level of attenuated positive psychotic symptoms at 6 and 12 months; effect sizes reported as standardized mean difference (SMD) and 95% CIs in mean follow-up scores between two compared interventions. The secondary outcome was treatment acceptability [reported as odds ratio (OR)]. NMAs were conducted for both primary and secondary outcomes. Treatments were cluster-ranked by surface under the cumulative ranking curve values for efficacy and acceptability. Assessments of biases, assumptions, sensitivity analyses and complementary pairwise meta-analyses for the primary outcome were also conducted.

Results: Overall, 1,707 patients from 14 studies (57% male, mean age = 20) were included, representing the largest evidence synthesis of the effect of preventive treatments on attenuated positive psychotic symptoms to date. In the NMA for efficacy, ziprasidone + Needs-Based Intervention (NBI) was found to be superior to

NBI (SMD = −1.10, 95% CI −2.04 to −0.15), Cognitive Behavioral Therapy-French and Morrison protocol (CBT-F) + NBI (SMD = −1.03, 95% CI −2.05 to −0.01), and risperidone + CBT-F + NBI (SMD = −1.18, 95% CI −2.29 to −0.07) at 6 months. However, these findings did not survive sensitivity analyses. For acceptability, aripiprazole + NBI was significantly more acceptable than olanzapine + NBI (*OR* = 3.73; 95% CI 1.01 to 13.81) at 12 months only. No further significant NMA effects were observed at 6 or 12 months. The results were not affected by inconsistency or evident small-study effects, but only two studies had an overall low risk of bias.

Conclusion: On the basis of the current literature, there is no robust evidence to favor any specific intervention for improving attenuated positive psychotic symptoms in CHR-P individuals.

Keywords: psychosis, risk, interventions, symptoms, network meta-analysis, treatments

INTRODUCTION

Indicated prevention in people at Clinical High Risk for Psychosis (hereafter CHR-P) (1) represents one of the first attempts to alter the course of the most severe psychiatric disorder and thereby improve the lives of many young people (2, 3). Recent meta-analytical evidence has suggested that it is potentially the only effective way to reduce the duration of untreated psychosis, which is a key factor determining outcomes (4). CHR-P individuals accumulate several risk factors for psychosis (5), leading to subtle symptoms (6) and functional impairments (7) that trigger help-seeking behaviors (8). CHR-P individuals have around 20% risk [eTable 4 from Fusar-Poli et al. (9)] of developing psychosis [but not any other non-psychotic disorder (10, 11)] at 2 years. After two decades of CHR-P research, the paradigm is at standstill (12). The principal limitations of knowledge are: (i) poor penetrance of detection strategies for identifying at-risk individuals (13, 14), (ii) the prognostic accuracy of CHR-P tools in clinical use (15) being substantially dependent on idiosyncratic sampling and recruitment strategies (16–20), and (iii) an unclear effect of preventive treatments. Our research group has previously addressed the first two limitations, and only more recently have we completed a meta-analysis that has investigated the consistency and magnitude of the effects of treatments to prevent psychosis in CHR-P individuals. We used a network meta-analytic approach, which allows head-to-head comparisons to be performed across different preventive treatments, and which is the recommended evidence synthesis method for informing treatment guidelines (21). The key result of our analysis was that there is no evidence to favor any specific preventive treatment for CHR-P individuals over any others (22). This finding is not completely surprising, given that all of the most recent trials in this area were negative (23–31). Therefore, currently, there is no convincing evidence that indicated interventions implemented in CHR-P individuals can effectively prevent the onset of psychosis. The impact of available preventive interventions on the underlying neurobiology that characterizes the CHR-P state and the onset of psychosis is similarly unclear (3). We have fully discussed these findings and the limitations of our analysis in our previous report (22).

Here, we complement our previous meta-analysis by focusing on outcomes other than the onset of new psychotic disorders. It is indeed apparent that CHR-P individuals may present with problems other than the development of psychosis at follow-up, such as the persistence of subthreshold psychotic symptoms (32). In particular, attenuated positive psychotic symptoms represent the defining features of the core CHR-P criteria. Meta-analytical evidence indicates that around 85% (95% CI 79% to 90%) of CHR-P individuals meet the intake criteria because of attenuated positive psychotic symptoms [see eFigure 1 in Fusar-Poli et al. (9)]. The severity and frequency of attenuated positive psychotic symptoms are carefully measured by experienced clinicians using specific semi-structured [and not self-administered (33)] CHR-P instruments (34). Investigating the effect of treatments on attenuated positive psychotic symptoms may also be associated with empirical research benefits. For example, it has been suggested that using continuous outcomes -such as attenuated positive psychotic symptoms- rather than the binary transition to psychosis may overcome the problems of arbitrary thresholds defining a categorical onset of psychosis (35). Investigating the impact of interventions on attenuated positive psychotic symptoms is also relevant for informing clinical guidelines. For example, the National Institute for Health and Care Excellence (NICE) guidelines recommend cognitive behavioral therapies (CBT) for presenting symptoms (36), but there is no clear evidence which can reliably support this recommendation. The other relevant outcome for CHR-P individuals is the acceptability of treatments. Given the relatively high proportion of false positives with respect to transition to psychosis, it is essential that treatments have a benign side effect profile, are well tolerated and acceptable to this patient group.

To address these gaps in knowledge, we present here a network meta-analysis investigating the consistency and magnitude of the effects of preventive treatments for reducing attenuated positive psychotic symptoms in CHR-P individuals, weighting the findings for acceptability. We focus on randomized controlled trials (RCTs) to avoid the selection biases associated with observational studies. Our primary aim was to test whether any specific treatments are any more or less effective (compared to any others) in improving attenuated positive psychotic symptoms

in CHR-P individuals, and to provide an evidence-based ranking of treatments on the basis of efficacy and acceptability. We intended that this work would contribute to the rigorous evidence-based assessment of the strengths and limitations of the CHR-P paradigm (12). Our overarching vision is that by understanding the limitations of current knowledge—which is an essential prerequisite to finding ways of overcoming them—the CHR-P field can advance with the development of refined approaches that may ultimately achieve an effective prevention of psychosis.

METHODS

Included Interventions

In a first step we listed the preventive interventions of interest. The current study included all RCTs involving non-pharmacological and/or pharmacological interventions administered to CHR-P individuals. We focused on the following types of treatments: CBT (different protocols), integrated psychological therapies, psychoeducational interventions, supportive counseling, family therapy, needs-based interventions (NBI), antipsychotic molecules (aripiprazole, ziprasidone, risperidone, olanzapine) and any novel/experimental therapeutics (D-serine and omega-3 fatty acids). Although these interventions had been defined *a priori*, we also allowed the inclusion of additional treatments that were emerging from the most recent literature search. In a second step, we carefully reviewed the available systematic reviews and meta-analyses to operationalize specific definitions of the preventive treatments in CHR-P individuals. This is an essential step to address heterogeneity across different types of interventions and to characterize the specific nodes that were composing our network. We defined each treatment component as indicated in the following paragraphs.

Needs-Based Interventions (NBI)

CHR-P individuals enrolled in clinical trials are traditionally young people who are experiencing subtle symptoms and functional impairment (7) and who are therefore seeking help for their problems (8). Accordingly, it is felt unethical to randomize them to a pure placebo or "no treatment" condition (37). In this scenario it is also difficult to provide an exact definition of "treatment as usual," because although treatment guidelines do exist (36), in reality treatment implementation is determined by local health service priorities, resources and configurations as well as availability of specialist training. We therefore decided on a pragmatic approach and adopted the operationalization of NBI provided by the founders of the CHR-P paradigm (38). This definition focuses on the symptoms and problems already presented by the help-seeking individual (39), and may encompass any of the following components: (a) needs-based supportive psychotherapy for problems with, for example, relationships, work or family; (b) case management for resolving issues with education, housing or employment; (c) brief family psychoeducation and general advice; (d) different types of medications other than antipsychotics; and (e) clinical monitoring alone or coupled with crisis management (38, 40).

Cognitive Behavioral Therapy, French and Morrison Protocol (CBT-F)

The CBT-F protocol (41), like the majority of CBT protocols, is grounded on the principles established by Beck (42). The intervention is problem-focused and time-limited, with treatment strategies selected based on the formulation of each patient's presenting problems but from a range of permissible, manualized strategies. Although each person's therapy will be tailored to their presenting needs, the core components include building engagement, collaborative goal-setting and formulation, normalizing psychotic-like experiences, evaluating core beliefs, and different types of behavioral experiments (41, 43).

Cognitive Behavioral Therapy, van der Gaag Protocol (CBT-V)

The protocol developed by van der Gaag et al. (44) is based on the French and Morrison protocol (41), which is then expanded by the addition of two novel components that target cognitive biases. The first additional component is education on dopamine system super-sensitivity and its relation to attenuated psychotic symptoms and exaggeration of cognitive biases, with the aim of normalizing aberrant perceptual experiences and reducing associated distress. The individual is taught how biases in cognition, such as selective attention, confirmation bias and jumping-to-conclusions contribute to the formation of delusions and paranoia (44). The second component involves exercises/behavioral experiments to correct these biases through examination of initial appraisals and testing of alternative explanations (45). Further aims of CBT-V include supporting school attendance and employment, improving relationships with friends and relatives, and if applicable, reducing cannabis use (44).

Integrated Psychological Interventions, Bechdolf Protocol (IPI)

The protocol developed by Bechdolf et al. (46) is a multi-component package of care. In addition to manualized and time-limited individual CBT-F (41), IPI also includes manualized group skills training, which focuses on scheduling and monitoring leisure activities, training in social skills, problem-solving and mastery of difficult situations, and developing "keeping well" strategies (46). The third component was computerized cognitive remediation to address thought and perception deficits (basic symptoms), and a final component included multi-family psychoeducation group sessions, which aimed to reduce interpersonal conflict and associated stress by helping family members better understand the CHR-P state (46, 47).

Family-Focused Therapy, Miklowitz Protocol (FFT)

The family-focused therapy (FFT) protocol by Miklowitz et al. (28) was originally developed for individuals with or at risk of bipolar disorder. The FFT was then adapted for CHR-P individuals, which has three broad stages. The first stage of FFT encompasses psychoeducation and the development of a patient-family prevention plan, which helps to increase understanding of the stressors contributing to attenuated positive

and negative symptoms, while also detailing coping strategies and behavioral activation goals. The second stage focuses on enhancing constructive patient-family communication, and the third stage consists of improving problem-solving skills (28).

Psychopharmacological Interventions

Pharmacological interventions included licensed medications, experimental pharmacotherapies as well as nutritional supplements.

Placebo

The placebo component was reserved for pharmacological placebos administered in the control arms of randomized controlled trials.

Node Composition

We carefully identified the specific interventions (as listed above) for each arm of every study, which were then linearly combined to compose the precise treatment "nodes" of our network. As discussed above, this definition of nodes is an essential prerequisite for performing a robust NMA that can be of clinical relevance. Each pharmacological treatment was assigned to its own node, but different dosages of the same molecule were categorized within the same node. While placebo was initially considered as a separate node from NBI, after performing sensitivity analyses to explore the effect of pooling them together, we decided to combine them in the same node (see below for details).

Search Strategy and Selection Criteria

The first step of our literature search involved systematic electronic searches in the Web of Science (which includes Web of Science Core Collection, BIOSIS Citation Index, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index and SciELO Citation Index) and Ovid/ PsychINFO databases, the Cochrane Central Register of Controlled Trials and the NHS Centre for Reviews and Dissemination (CRD), using the following keywords: (risk OR prodromal OR prodrom* OR ultra-high risk OR clinical high risk OR high risk OR genetic high risk OR at risk mental state OR risk of progression OR progression to first-episode OR prodromally symptomatic OR basic symptoms) AND (psychosis) AND (RCT OR randomized controlled trial OR placebo controlled trial OR trial). The searches were conducted up to 18th July 2017 and no language restrictions were applied. In a second step, we used Scopus/Web of Science to screen the reference lists of articles identified in the previous step and those of existing systematic reviews and meta-analyses. For comprehensiveness, we also searched the reference lists of relevant clinical guidelines. In a third step, we looked for published and unpublished material in relevant conference proceedings, trial registries (e.g., https://clinicaltrials.gov) or regulatory agencies. The OpenGrey database (http://www.opengrey.eu) was used to identify unpublished material from the gray literature.

The above search strategies led us to identifying potential abstracts of interest. The abstracts were then screened for potential inclusion and those that survived this initial filter were downloaded as full-text articles. These were then carefully inspected against the full inclusion and exclusion criteria which are described below.

In line with the PRISMA guidance, two independent researchers conducted the literature search, study selection and data extraction (48). During the above steps, disagreement between extractors was addressed through discussion with a third researcher until consensus was obtained. We defined the specific inclusion and exclusion criteria to ensure that the population represented in the final database would be broadly representative of the target CHR-P population as a whole (49).

Our inclusion criteria were (a) being an original article, abstract or pilot study; (b) being a randomized controlled trial (including cluster randomized trials, but excluding cross-over studies); (c) being designed as blinded (either single- or double-blind); (d) being conducted in CHR-P individuals with CHR-P criteria ascertained through the use of internationally validated psychometric assessments, i.e., the Comprehensive Assessment of At-Risk Mental States (CAARMS) (6), the Structured Interview for Psychosis-risk Syndromes (SIPS) (50), the Positive and Negative Syndrome Scale (PANSS) (51), the Brief Psychiatric Rating Scale (BPRS) (52), or the Early Recognition Inventory (ERIraos) (53); (e) comparing specific preventive interventions as defined in the sections above; (f) providing sufficient data to perform meta-analytic computation; (g) providing a sample size of 10 or greater (54).

Our exclusion criteria were defined as (a) being a review or reporting non-original data; (b) lacking at least two compared groups, such as open-label trials in a single group of CHR-P patients exposed to treatment; (c) investigating patient samples affected with an established first-episode psychosis or any at-risk group other than CHR-P samples; (d) lacking sufficient data needed to perform the essential meta-analytical computations; (e) design lacking proper randomization, such as quasi-randomization or observational naturalistic studies - however, studies that were initially conceived and designed as blinded but could not maintain blinding during follow-up (e.g., for psychological interventions) were not excluded; (f) including a sample size smaller than 10 (i.e., $N = 9$ or less); (g) presenting overlapping data (i.e., for the same outcome at the same time point as data that was already included)-in the case of overlapping data/samples, we preferred the data relating to the largest sample size.

Outcome Measures and Data Extraction

Due to the variable effect of time on clinical outcomes in these samples (9, 55), analyses for time-dependent outcomes were conducted. The primary outcome (efficacy) was the level of attenuated positive psychotic symptoms at follow-up, indexed by the relevant subscales of validated assessments, such as the PANSS, CAARMS, BPRS and SIPS. For each arm of every study, we extracted the mean and standard deviation (SD) of these scores at 6 and 12 month follow-up time points. Where studies did not report sufficient data to extract the primary outcome, we used DigitizeIt software (http://www.digitizeit.de/) to extract data presented graphically (means and 95% confidence intervals (CIs) for each follow-up time point). When necessary,

SDs were back-calculated using standard formulae. If none of the aforementioned were available, we estimated follow-up data using information available from the published paper and using assumptions established in previous literature. Sample sizes were based on the numbers randomized to each arm.

A high benefit-to-harm ratio is essential when adopting preventive strategies that may lead to the unnecessary treatment of false positives. We therefore selected the acceptability of interventions (discontinuation due to any cause) as our secondary outcome measure. In line with previous authoritative publications, we defined the acceptability of interventions as the number of participants who dropped out of each arm for any reason following randomization, over those randomized at baseline (56–58).

In order to describe our population, assess the transitivity assumption (see below), address the risk of bias and conduct meta-regression analyses, we also extracted details on the first author and year of publication of each trial, country where the trial was conducted, types of outcomes reported, definitions of intervention and control arms (in line with the treatment components described above), trial design, risk of bias assessment, duration of each intervention and follow-up, sample size, mean age, percent male, and the psychometric CHR-P instrument used to ascertain attenuated positive psychotic symptoms.

Risk of Bias

The assessment of bias is of paramount importance for rigorously interpreting the results of evidence synthesis studies and for testing their robustness. We used the Cochrane Risk of Bias tool (59) to classify the risk of bias in each study using *a priori* defined criteria. Using these standardized criteria, we evaluated whether each trial was at high, low or unclear risk of bias across six specific domains. These included random sequence generation, allocation concealment, blinding of participants and study personnel, blinding of outcome assessments, incomplete outcome data, and selective outcome reporting. Once these domains were assessed, the Cochrane Risk of Bias tool allowed production of an overall risk of bias classification of high, low or unclear. The overall rating of low risk was assigned when none of the six domains were found to be at high risk and if three or less domains were found to be at unclear risk. The overall rating of moderate risk was assigned when one domain was found to be at high risk; or no domains were found to be at high risk but four or more were found to be at unclear risk. In all other cases, the trial was classified as having an overall high risk of bias (60).

Statistical Analysis

Network Meta-Analysis

Frequentist NMAs were conducted for both primary (attenuated positive psychotic symptoms) and secondary (acceptability) outcomes at 6 and 12 months using the *network* package in STATA (version SE 14.2). Effect sizes for the primary outcome were calculated and reported as the standardized mean difference Hedges' adjusted *g* (SMD) and 95% CIs in mean follow-up scores between two compared interventions, using the pooled SD at follow-up (61). Follow-up data are considered preferable when measuring continuous outcomes that are difficult to measure (62). Effect sizes for the secondary outcome were reported as odds ratio (OR) and 95% CIs. We first constructed network plots - for each outcome- to ensure that the geometry of the networks were sufficiently connected (63, 64). We then performed a NMA assuming consistency and a common heterogeneity across all comparisons in the network. This allowed us to derive a single summary treatment effect (SMD for attenuated positive psychotic symptoms; OR for acceptability) for every possible pairwise comparison of treatments. This summary effect draws on all evidence from the network of trials, including direct and indirect evidence. Correlations in effect sizes induced by multi-arm trials were accounted for (63, 65). The resulting relative SMDs or ORs with 95% CIs for each pair of treatments were reported in league tables (66). Statistical significance was set at $p < 0.05$.

When performing NMA it is possible to rank an outcome of interest using the Surface Under the Cumulative RAnking curve (SUCRA) procedure. Such an approach allows integration of both the location and the variance of any relative effect on the outcome of interest (67). In simple terms, the SUCRA procedure summarizes the overall ranking of each intervention through a single number ranging from 0 to 100% (68). In this manuscript, the higher the SUCRA value, the higher the likelihood that an intervention will be in the top rank, and vice versa (68). In line with our objective, we performed cluster ranking (63, 67) of the SUCRA values for attenuated positive psychotic symptoms and acceptability (at 6 and 12 months, separately) and presented the results in two-dimensional plots (64). These plots aid visualization of the relative balance between a treatment's ranking across different outcomes, and show the clustering of treatments into meaningful groups as determined by hierarchical cluster analysis (63, 64).

Consistency in a network refers to the equivalence of direct and indirect estimates of the same treatment comparison pairs, and can be investigated in each closed loop of evidence (66). We assessed this assumption by calculating an inconsistency factor along with 95% CIs (truncated at 0) and associated *p*-values for each closed loop of the primary outcome (63). Inconsistency was defined as disagreement between direct and indirect evidence, with 95% CIs for inconsistency factors excluding zero. Because the loop-specific approach focuses on local inconsistency and has low power, we also tested a full design-by-treatment model (69) for the primary outcome to evaluate global inconsistency. This entailed performing a NMA under the inconsistency model and using the χ^2-test to estimate the statistical significance of all possible inconsistencies in the networks (70).

Transitivity was examined by assessing the distributions of potential effect modifiers across comparisons in the networks. These effect modifiers encompassed the following items: percent male, age, percent exposed to antipsychotic medications at baseline, type of blinding and publication year.

The presence of small-study effects was assessed by visual inspection of comparison-adjusted funnel plots (71). In this analysis we used NBI (or when not available, CBT-F + NBI) as the reference. We assumed that small-study effects, if present, would be expected to exaggerate the effectiveness of the "active" (or newer/experimental) treatment, rather than NBI or CBT-F

+ NBI, which currently represent the most widely implemented interventions for this patient group.

Complementary Analyses

Sensitivity analyses were performed to address the impact of study quality and our data analysis strategy. Specifically, we repeated the NMA analyses on attenuated positive psychotic symptoms using only: (a) studies with a low risk of bias for the blinding of attenuated positive psychotic symptom assessments; (b) studies whose meta-analytical data (i.e., mean and SD of attenuated positive psychotic symptoms) were not estimated using assumptions established in previous literature; and (c) published trials only (i.e., excluding conference proceedings). In addition, we repeated the NMA after pooling NBI and placebo nodes and after pooling different types of antipsychotic molecules. To ensure that the use of follow-up scores did not unduly influence our results, we repeated the analyses using SMD calculated from change score and pooled baseline SD, which is recommended when a full ANCOVA model is not feasible (62). Furthermore, we complemented the sensitivity analyses through network meta-regression analyses. These were planned only when substantial heterogeneity was observed and when at least 10 independent trials were available (72) for each outcome of interest. These meta-regressions were planned to investigate the potential impact of the different CHR-P psychometric instruments used for measuring attenuated positive psychotic symptoms (34).

For the primary outcome, we also conducted conventional pairwise meta-analyses (random effects model) of every direct treatment comparison using the *metan* package in STATA. The random effects meta-analyses were stratified by (a) follow-up time (6 or 12 months), and (b) pairwise intervention comparisons (i.e., each type of treatment vs. its control was treated as a meta-analysis, no overall summary effect computed across comparisons of different treatments). The resulting meta-analytic SMDs together with 95% CIs and measures of heterogeneity (I^2) were calculated and presented in tables. When pairwise groups had more than three contributing studies, we performed leave-one-out sensitivity analyses to explore the robustness of the results to influential individual studies.

RESULTS

Characteristics of Trials and Patients

Our initial literature search identified 1,556 references. However, most of them did not report on RCTs in CHR-P individuals. As indicated in **Figure 1**, 49 of them were eventually downloaded and fully inspected against the inclusion and exclusion criteria, which resulted in a final sample of 14 studies. We found only three, three, two and two trials reporting the outcome data of interest at 18, 24, 36, and >36 month time points, respectively. Consequently, the current meta-analysis focuses only on the 6 and 12 month time points. The 14 studies used in the analyses contributed data on a total of 1707 patients, with a mean age of 20.1 ± 2.9 years, and of whom 57% were male (**Table 1**). The mean sample size was 122 (range 44–304). Five studies were conducted in North America, five in Europe, three in Australia and one was multi-national. Two trials adopted a three-arm design while all of the others employed a two-arm design. Two of the included studies were identified from conference proceedings and gray literature/clinical trial databases (24, 30). Two studies had a treatment duration of <6 months, eight of 6 months, and four of 12 months. Three of the 12 trials that reported enough information to identify the source of sponsorship or funding acknowledged pharmaceutical company involvement. The SIPS was the most common assessment used for measuring attenuated positive psychotic symptoms ($N = 6$). Only two studies had an overall low risk of bias (30, 40), four had unclear risk (24, 26, 27, 73) and the remaining eight had high risk; the full risk of bias assessment is presented in **Figure 2**.

For the primary outcome, eight studies provided data for both 6 and 12 month networks, three only provided data for 6 months, and another 3 only for 12 months, resulting in 11 studies contributing data for the 6 month analysis, and 11 to the 12 month analysis. For the 6 month analysis, 11 studies ($N = 1459$) provided data on 15 direct comparisons between 8 different treatment nodes (**Figure 3A**). For the 12 month analysis, 11 studies ($N = 1483$) provided data on 15 direct comparisons between 7 different treatment nodes (**Figure 3B**).

At 6 months, seven studies provided the required follow-up symptom data directly or indirectly, two provided means and SD graphically (28, 40), and for two studies symptom data were estimated on the basis of available data and assumptions established in previous literature (27, 30). At 12 months, nine studies provided the required data directly or indirectly, one provided data graphically (40), and for one study symptom data were estimated on the basis of available data and assumptions established in previous literature (27).

All studies, except one (38), provided data for the secondary outcome (acceptability) at both 6 and 12 months. Network plots for the acceptability outcome were the same as those for the primary outcome (**Figure 3**).

Pairwise Meta-Analysis

Pairwise meta-analysis results for the primary outcome are presented in **Table 2**. Only three pairwise intervention vs. control groups had two or more studies: CBT-F + NBI vs. NBI; omega-3 + NBI vs. NBI; and risperidone + CBT-F + NBI vs. NBI. The remaining pairwise intervention vs. control groups were composed of single studies.

At 6 months, there was no significant difference between CBT-F + NBI vs. NBI alone (SMD = −0.06, 95% CI −0.26 to 0.13; 5 studies, $N = 652$). However, there was meta-analytical evidence of a greater reduction in attenuated positive psychotic symptoms in CBT-F + NBI vs. NBI alone at 12 months (SMD = −0.22, 95% CI −0.37 to −0.07; 6 studies, $N = 712$). Leave-one-out sensitivity analyses showed that this effect was dependent on the presence of one study (74). When this study was removed, the combined effect at 12 months became non-significant (SMD = −0.12, 95% CI −0.32 to 0.08; 5 studies, $N = 424$). The non-significant summary effect at 6 months did not become significant throughout any iteration of the leave-one-out analyses.

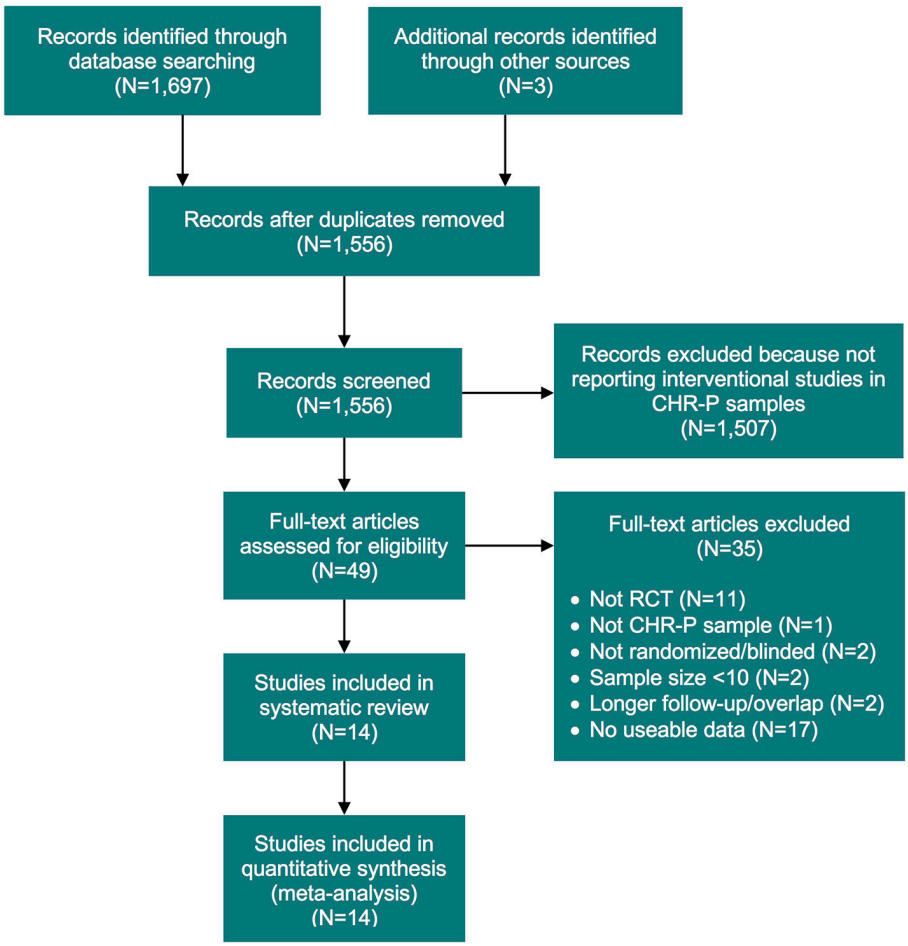

FIGURE 1 | PRISMA flow chart of the study selection process. CHR-P, clinical high risk for psychosis; RCT, randomized controlled trial.

Two studies compared omega-3 + NBI to NBI alone, but both 6 and 12 month summary effect estimates were not significant (6 month SMD = −0.48, 95% CI −1.62 to 0.67, 2 studies, $N = 385$; 12 month SMD = −0.38, 95% CI −1.38 to 0.63, 2 studies, $N = 385$). Significant heterogeneity was detected between these two studies at both 6 ($I^2 = 95\%$, $p < 0.001$) and 12 ($I^2 = 94\%$, $p < 0.001$) month time points. Statistical investigation of potential sources of heterogeneity using meta-regression was precluded by the limited number of studies.

Combined therapy with risperidone + CBT-F + NBI was not significantly different from NBI alone at either 6 (SMD = 0.02, 95% CI −0.33 to 0.37, 2 studies, $N = 130$) or 12 months (SMD = 0.00, 95% CI −0.38 to 0.38, 2 studies, $N = 130$). While available data on all further pairwise treatments vs. controls are listed in **Table 2** for completeness, they represent single studies and thus cannot be considered meta-analytic results.

Network Meta-Analysis – Effect on Attenuated Positive Psychotic Symptoms

Results of the NMA are presented in **Tables 3**, **4**. At 6 months, ziprasidone + NBI was found to be significantly more effective at reducing attenuated positive psychotic symptoms compared to NBI alone (SMD = −1.10, 95% CI −2.04 to −0.15); compared to CBT-F + NBI (SMD = −1.03, 95% CI −2.05 to −0.01); and compared to risperidone + CBT-F + NBI (SMD = −1.18, 95% CI −2.29 to −0.07). There were no other significant effects of any one intervention over any others (**Table 3**). Using NBI as a comparator, the relative treatment effect estimates (all SMD < 0 favor the given treatment) at 6 months were: ziprasidone + NBI (SMD = −1.10, 95% CI −2.04 to −0.15); omega-3 + NBI (SMD = −0.42, 95% CI −1.01 to 0.16); aripiprazole + NBI (SMD = −0.18, 95% CI −0.90 to 0.53); family-focused therapy + NBI (SMD = −0.41, 95% CI −1.22 to 0.41); CBT-F + NBI (SMD = −0.07, 95% CI −0.44 to 0.31); D-serine + NBI (SMD = −0.10, 95% CI −1.05 to 0.84); and risperidone + CBT-F + NBI (SMD = 0.08, 95% CI −0.50 to 0.67).

At 12 months, there was no evidence that any one intervention was superior over any others, with all 95% CIs crossing zero (**Table 4**). Using NBI as a comparator, the relative treatment effect estimates (all SMD < 0 favor the given treatment) at 12 months were: olanzapine + NBI (SMD = −0.53, 95% CI −1.28 to 0.22); omega-3 + NBI (SMD = −0.30, 95% CI −0.77 to 0.17);

TABLE 1 | Characteristics of included studies.

Study	Study arms (N)	Total N	Network inclusion	Treatment duration (months)	Follow-up time points (months)	% male	Mean age	Measure of symptoms	Study design	Country	% exposed to antipsychotics at baseline
Addington et al. (37)	CBT-F + NBI [27] NBI [24]	51	6, 12	6	6, 12, 18	71	20.9	SIPS	SB-RCT	Canada	0
Amminger et al. (40)	Omega-3 + NBI [41] NBI [40]	81	6, 12	3	6, 12, 84	33	16.4	PANSS	DB-RCT	Austria	0
Bechdolf et al. (46, 75)	IPI [63] NBI [65]	128	12	12	6, 12, 18, 24	63	26.0	PANSS	SB-RCT	Germany	0
Bechdolf et al. (24)	ARI + NBI [96] NBI [55] CBT-F + NBI [129]	280	6, 12	12	6, 12	66	24.4	SIPS	SB-RCT	Germany	3.4
Kantrowitz et al. (26)	D-serine + NBI [20] NBI [24]	44	6	4	4	66	19.4	SIPS	DB-RCT	US	11.4
McGlashan et al. (73)	OLA + NBI [31] NBI [29]	60	12	12	12, 24	65	17.7	SIPS	DB-RCT	US, Canada	10
McGorry et al. (38)	RIS + CBT-F + NBI [31] NBI [28]	59	6, 12	6	6, 12, 36–48	58	20.0	BPRS	SB-RCT	Australia	0
McGorry et al. (27)	Omega-3 + NBI [153] NBI [151]	304	6, 12	6	6, 12	46	19.2	BPRS	DB-RCT	Multi-national	0
Miklowitz et al. (28)	FFT + NBI [66] NBI [63]	129	6	6	6	57	17.4	SIPS	SB-RCT	US, Canada	20.9
Morrison et al. (43)	CBT-F + NBI [37] NBI [23]	60	12	6	6, 12, 36	69	22.0	PANSS	SB-RCT	UK	0
Morrison et al. (74)	CBT-F + NBI [144] NBI [144]	288	6, 12	6	6, 12, 18, 24	63	20.7	CAARMS	SB-RCT	UK	0
Stain et al. (29)	CBT-F + NBI [30] NBI [27]	57	6, 12	6	6, 12	40	16.3	CAARMS	SB-RCT	Australia	0
Woods et al. (30, 31)	ZIP + NBI [24] NBI [27]	51	6	6	6	64	22.3	SIPS	DB-RCT	US	0
Yung et al. (76) and McGorry et al. (77)	CBT-F + NBI [44] RIS + CBT-F + NBI [43] NBI [28]	115	6, 12	12	6, 12	39	18.1	BPRS	SB-RCT	Australia	0

CBT-F, cognitive behavioral therapy (French and Morrison protocol); NBI, eeds-based interventions (including placebo); IPI, integrated psychological interventions; ARI, aripiprazole; OLA, olanzapine; RIS, risperidone; FFT, family-focused therapy; ZIP, ziprasidone; SIPS, Structured Interview for Psychosis-risk Syndromes; PANSS, Positive and Negative Syndrome Scale; BPRS, Brief Psychiatric Rating Scale; CAARMS, Comprehensive Assessment of At-Risk Mental States; SB-RCT, single-blind randomized controlled trial; DB-RCT, double-blind randomized controlled trial.

aripiprazole + NBI (SMD = −0.23, 95% CI −0.78 to 0.33); CBT-F + NBI (SMD = −0.15, 95% CI −0.43 to 0.13); risperidone + CBT-F + NBI (SMD = −0.04, 95% CI −0.52 to 0.44); and integrated psychological interventions (SMD = 0.20, 95% CI −0.45 to 0.84).

Inconsistency and Small-Study Effects

There was no statistically significant inconsistency in the 6 or 12 month networks. The 95% CIs for all inconsistency factors were compatible with zero inconsistency. However, it is important to note that only two loops were available at both 6 and 12 months, which may have limited our ability to detect inconsistency. When we used the design-by-treatment interaction model, there was no evidence for significant inconsistency in the 6 ($p = 0.92$) or 12 month ($p = 0.92$) networks.

Visual inspection of comparison-adjusted funnel plots suggested no clear small-study effects (publication biases), with a regression line almost flat at 6 months (Figure S1A in Supplementary Material) and completely flat at 12 months (Figure S1B in Supplementary Material).

Sensitivity Analyses for NMA of Primary Outcome

We tested the robustness of the core NMA findings (that ziprasidone + NBI is superior to NBI alone, CBT-F + NBI, and risperidone + CBT-F + NBI at 6 months) through various sensitivity analyses. At 6 months, two studies (27, 30) were based on estimated follow-up data, one of which was the single ziprasidone + NBI vs. NBI study (30). Repeating the analyses after removal of the latter study (30) inherently meant that there was now no ziprasidone + NBI node and all estimates were non-significant. Removal of the other study -by McGorry et al (27)- did not affect the current results at 6 or 12 months; however, one change of note is that at 12 months, CBT-F + NBI became significantly more effective than NBI, which is interesting in light of the pairwise significance of CBT-F + NBI vs. NBI at 12 months, and lack thereof in the main NMA analyses.

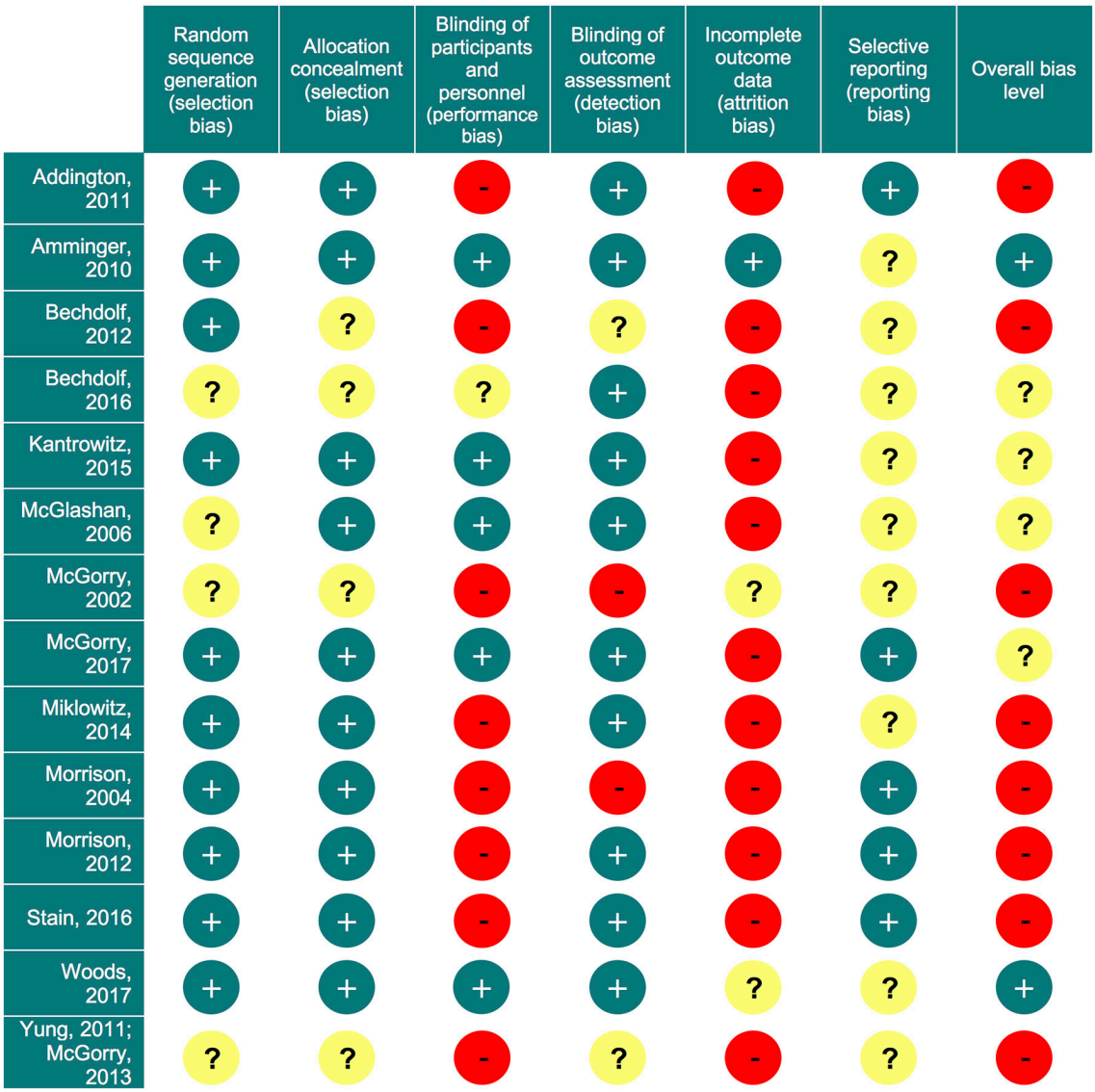

FIGURE 2 | Risk of bias summary. Review authors' judgements about each risk of bias item for each included study. Green circles (+) indicate low risk of bias; yellow circles (?) indicate unclear risk; red circles (–) indicate high risk of bias.

The ziprasidone + NBI results were not robust to the removal of two studies (38, 76) at high or unclear risk of bias for the blinding of outcome assessments, which resulted in only the ziprasidone + NBI vs. NBI comparison remaining significant. Repeating the analyses after removing unpublished studies (24, 30), pooling together different antipsychotic molecules, and using change scores instead of follow-up scores all abolished the ziprasidone + NBI results. Repeating the analyses treating NBI + placebo as a separate node to NBI had some effect on the NMA estimates at 6 months (ziprasidone + NBI was now superior only to NBI + placebo) and had no effect at 12 months; we therefore used the pooled NBI + placebo in the main analysis (**Tables 1–4**, **Figures 3**, **4**). There were too few studies to allow robust meta-regression analyses on the type of instruments used to measure attenuated positive psychotic symptoms.

Network Meta-Analysis – Effect on Acceptability

There were no significant differences in acceptability between any treatments at 6 months (**Table 3**). At 12 months, aripiprazole + NBI was significantly more acceptable than olanzapine + NBI (*OR* = 3.73; 95% CI 1.01 to 13.81). There were no further significant differences at 12 months (**Table 4**). However, at both time points, the 95% CIs for the comparisons were often very wide, indicating substantial imprecision in the estimates.

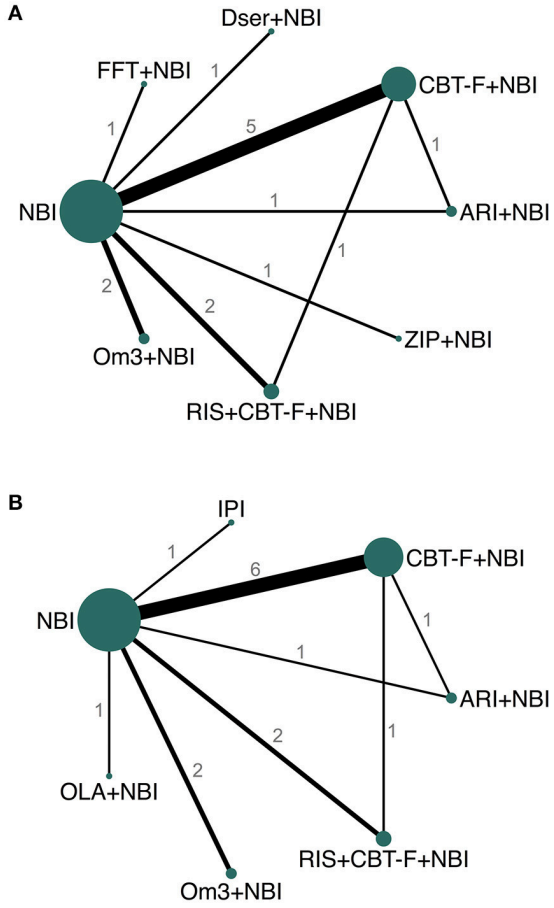

FIGURE 3 | Network plots of direct comparisons at 6 **(A)** and 12 **(B)** months. The width of the lines is proportional to the number of trials comparing each pair of treatments and the size of each node is proportional to the number of studies testing the specific treatment. Numbers represent the number of studies contributing data to each comparison. ARI, aripiprazole; NBI, needs-based interventions (including placebo); CBT-F, cognitive behavioral therapy (French and Morrison protocol); Dser, D-serine; FFT, family-focused therapy; Om3, omega-3 fatty acids; RIS, risperidone; ZIP, ziprasidone; IPI, integrated psychological interventions; OLA, olanzapine.

Network Meta-Analysis – Cluster Ranking for Attenuated Positive Psychotic Symptoms and Acceptability

The cluster ranking plots of SUCRA values for attenuated positive psychotic symptoms (efficacy) and acceptability are illustrated in **Figure 4A** (for 6 months) and **Figure 4B** (for 12 months). However, it should be noted that although the treatments were cluster ranked, there was no statistically significant difference between any treatments (with the exception of ziprasidone + NBI) in the main network meta-analysis results (see **Tables 3**, **4** for details).

Three distinct clusters were found in the cluster ranking at 6 months (**Figure 4A**). Notably, while ziprasidone + NBI had the highest SUCRA for efficacy (94%), it was also the most poorly tolerated, having the lowest SUCRA value for acceptability (23%). In a second cluster, omega-3 + NBI and family-focused therapy + NBI had similar SUCRA scores for efficacy (67% and 63%, respectively), however, they differed markedly in their SUCRA for acceptability; family-focused therapy + NBI had the highest acceptability SUCRA of all treatments (70%), while that of omega-3 + NBI was mid-range (49%). The third cluster comprised the remaining treatments, whose SUCRA values for efficacy were all below 50%, but whose acceptability SUCRAs varied from 62% for aripiprazole + NBI, to 37% (the worst) for NBI.

At 12 months, four distinct clusters were found (**Figure 4B**). Similar to above, while olanzapine + NBI was ranked highest of all treatments for efficacy SUCRA (82%), it also scored worst for acceptability (13%). A second cluster, with a more balanced profile of efficacy and acceptability SUCRA values, comprised aripiprazole + NBI, omega-3 + NBI and CBT-F + NBI. Of these, omega-3 + NBI had the highest SUCRA value in terms of efficacy (70%) but lower acceptability (57%), aripiprazole + NBI had slightly lower efficacy (60%) but the highest acceptability of all treatments (91%), and CBT-F + NBI had mid-range values for both outcomes. NBI and risperidone + CBT-F + NBI were found in a third, intermediate cluster with low mid-range SUCRA values. The final cluster was composed of integrated psychological interventions, with SUCRA values of 19% and 26% for efficacy and acceptability, respectively.

DISCUSSION

To the best of our knowledge, this is the first network meta-analysis to have explored the effect of preventive treatments on attenuated positive psychotic symptoms in CHR-P individuals. Focusing exclusively on RCTs to minimize selection biases, we included a total of 14 non-overlapping studies, for a total database of 1,707 CHR-P individuals, representing the largest evidence synthesis of this topic to date. By using the most updated evidence we defined two networks at 6 and 12 months, on which we performed the core analyses. These two networks included 8 and 7 nodes, respectively. There were not enough studies to generate networks beyond these time points. Overall, our network meta-analyses indicated no robust evidence of superior efficacy for any specific intervention on attenuated positive psychotic symptoms at any time point, with the exception of ziprasidone + NBI, which was superior to NBI alone, CBT-F + NBI, and risperidone + CBT-F + NBI. However, the evidence specifically relating to ziprasidone + NBI was based on a single study only and did not survive sensitivity analyses. The results were not affected by inconsistency or evident small-study effects (publication biases).

The main finding of the current study is that there is a lack of evidence to favor specific effective interventions for reducing attenuated positive psychotic symptoms in CHR-P individuals. While ziprasidone + NBI demonstrated some superiority in the 6 month network meta-analyses, these results are not robust. First, the efficacy of ziprasidone + NBI comes from only one as-yet unpublished study. Second, the results did not survive most of the sensitivity analyses. Finally, in the cluster ranking, it was clear that while ziprasidone + NBI was the most efficacious in reducing

TABLE 2 | Pairwise meta-analytic results for attenuated psychotic symptoms at 6 and 12 months.

Time point; months	Treatment condition	Control condition	Number of studies (references)	Total N		SMD (*g*)	Lower 95%CI	Upper 95%CI	Heterogeneity		
				Treatment	Control				I^2	Q	P
6	ARI + NBI	NBI	1 (24)	96	55	−0.22	−0.56	0.11	–	0.00	–
	ARI + NBI	CBT-F+NBI	1 (24)	96	129	−0.06	−0.33	0.20	–	0.00	–
	CBT-F + NBI	NBI	5 (24, 29, 37, 74, 76)	374	278	−0.06	−0.26	0.13	24.2	5.27	0.26
	Dser + NBI	NBI	1 (26)	20	24	−0.10	−0.70	0.49	–	0.00	–
	FFT + NBI	NBI	1 (28)	66	63	−0.41	−0.76	−0.06	–	0.00	–
	Om3 + NBI	NBI	2 (27, 40)	194	191	−0.48	−1.62	0.67	94.8	19.4	<0.001
	RIS + CBT-F + NBI	CBT-F+NBI	1 (76)	43	44	0.37	−0.05	0.80	–	0.00	–
	RIS + CBT-F + NBI	NBI	2 (38, 76)	74	56	0.02	−0.33	0.37	0.00	0.72	0.40
	ZIP + NBI	NBI	1 (30)	24	27	−1.10	−1.69	−0.50	–	0.00	–
12	ARI + NBI	NBI	1 (24)	96	55	−0.22	−0.55	0.12	–	0.00	–
	ARI + NBI	CBT-F+NBI	1 (24)	96	129	−0.08	−0.34	0.18	–	0.00	–
	CBT-F + NBI	NBI	6 (24, 29, 37, 43, 74, 77)	411	301	**−0.22**	**−0.37**	**−0.07**	0.00	3.27	0.66
	IPI	NBI	1 (46)	63	65	0.20	−0.15	0.54	–	0.00	–
	OLA + NBI	NBI	1 (73)	31	29	−0.53	−1.05	−0.02	–	0.00	–
	Om3 + NBI	NBI	2 (27, 40)	194	191	−0.38	−1.38	0.63	93.5	15.49	<0.001
	RIS + CBT-F + NBI	CBT-F+NBI	1 (77)	43	44	−0.07	−0.49	0.35	–	0.00	–
	RIS + CBT-F + NBI	NBI	2 (38, 77)	74	56	0.00	−0.38	0.38	16.2	1.19	0.28

Underlined bold text within the SMD and 95% CI columns indicates statistically significant meta-analytic treatment effect. SMD below 0 favors the given treatment condition. ARI, aripiprazole; NBI, needs-based interventions (including placebo); CBT-F, cognitive behavioral therapy (French & Morrison protocol); Dser, D-serine; FFT, family-focused therapy; Om3, omega-3 fatty acids; RIS, risperidone; ZIP, ziprasidone; IPI, integrated psychological interventions; OLA, olanzapine. Dashes (–) indicate no heterogeneity estimate due to having only one contributing study (and thus cannot be considered a true meta-analytic result).

attenuated positive symptoms, it was poorly tolerated with the lowest ranking for acceptability. Similarly, the only significant result in pairwise analyses was for CBT-F + NBI vs. NBI at 12 months. Again, this was found to be reliant on the inclusion of one particular study (74) in sensitivity analyses. Given that the data relating to the CBT-F + NBI element are identical in both the pairwise and network meta-analyses, the driving factor for the disparity (in significance of CBT-F + NBI vs. NBI in pairwise vs. network meta-analyses) likely emerges from the additional data about NBI that the NMA had gained from the rest of the network (i.e., the relative effectiveness of NBI as derived indirectly from the other -direct- comparisons). Support for this explanation comes from the finding that, when one particular study (27) was removed from the 12 month network (in sensitivity analyses), the CBT-F + NBI vs. NBI comparison became significant. Inspection of the data for this removed study (27) showed that it had the largest NBI arm ($N = 151$) of all trials, and although the study-specific SMD was not significant, the SMD was favoring NBI over the comparative intervention (omega-3 + NBI). This suggests that the relative effectiveness of NBI may have been underestimated by the direct (pairwise) CBT-F + NBI vs. NBI estimates compared to the NMA-derived estimates.

Overall, our negative results are concordant with several lines of evidence pointing toward ineffective treatments for CHR-P individuals. Beyond the lack of evidence for specific treatments reducing the risk of developing psychosis -as determined by our earlier study (22)-, another recently published network meta-analysis found no evidence that any treatments were better than any others in improving attenuated negative symptoms in CHR-P individuals (78, 79). The lack of impact on attenuated negative symptoms is in line with meta-analytical evidence showing that full-blown negative symptoms are refractory to any kind of treatment (72). More to the point, there is not even evidence that current preventive treatments can ameliorate clinical outcomes such as functional level (80–83), depressive comorbidities (83), distress (81) and quality of life (81, 83) in CHR-P individuals. It is possible that the lack of evidence for effective treatments to reduce transition to psychosis may be secondary to low statistical power for testing this outcome. In turn, this can be caused by the recruitment strategies adopted by recent RCTs that have focused on individuals that were poorly risk enriched, causing a dilution of the final risk for psychosis (23). On the contrary, the lack of evidence for effects on attenuated positive psychotic symptoms cannot simply be attributed to low statistical power. Rather, it is possible that the available treatments are not disease-modifying because they are not targeting the core pathophysiological processes underlying the onset of psychosis in CHR-P individuals (3). It is also possible that effective preventive treatments do exist, but we are currently unable to detect them because of the large noise and between-subject heterogeneity that is observed. For example, the level of attenuated positive psychotic symptoms varies considerably across different CHR-P subgroups. We have previously found that CHR-P individuals meeting the short-lived psychotic episode subgroup have the highest risk of developing psychosis (about 40–50% at 2 years) (9, 84), those meeting the attenuated psychotic symptoms subgroup have an intermediate risk (about 20% at 2 years) (9), and those meeting the genetic risk subgroup have a low risk (about

TABLE 3 | Network meta-analytic relative treatment effects for efficacy and acceptability at 6 months.

ZIP + NBI	1.77 (0.32,9.79)	2.63 (0.42,16.68)	2.22 (0.39,12.75)	1.83 (0.24,13.78)	1.79 (0.40,8.07)	1.55 (0.38,6.27)	2.28 (0.34,15.09)
−0.67 (−1.78,0.44)	**Om3 + NBI**	1.49 (0.31,7.07)	1.26 (0.30,5.30)	1.04 (0.18,6.01)	1.01 (0.33,3.13)	0.88 (0.33,2.35)	1.29 (0.26,6.44)
−0.69 (−1.94,0.56)	−0.01 (−1.02,0.99)	**FFT + NBI**	0.84 (0.17,4.15)	0.69 (0.11,4.59)	0.68 (0.18,2.56)	0.59 (0.18,1.96)	0.87 (0.15,4.98)
−0.91 (−2.10,0.27)	−0.24 (−1.16,0.68)	−0.23 (−1.31,0.86)	**ARI + NBI**	0.82 (0.14,4.93)	0.80 (0.28,2.28)	0.70 (0.25,1.98)	1.03 (0.21,5.02)
−0.99 (−2.33,0.35)	−0.32 (−1.43,0.79)	−0.30 (−1.55,0.94)	−0.08 (−1.26,1.11)	**Dser + NBI**	0.98 (0.21,4.65)	0.85 (0.20,3.63)	1.25 (0.18,8.60)
−1.03 (−2.05,−0.01)	−0.36 (−1.05,0.34)	−0.34 (−1.24,0.56)	−0.11 (−0.82,0.59)	−0.04 (−1.05,0.98)	**CBT-F + NBI**	0.87 (0.50,1.51)	1.27 (0.36,4.46)
−1.10 (−2.04,−0.15)	−0.42 (−1.01,0.16)	−0.41 (−1.22,0.41)	−0.18 (−0.90,0.53)	−0.10 (−1.05,0.84)	−0.07 (−0.44,0.31)	**NBI**	1.47 (0.41,5.25)
−1.18 (−2.29,−0.07)	−0.50 (−1.33,0.32)	−0.49 (−1.49,0.51)	−0.26 (−1.16,0.63)	−0.18 (−1.30,0.93)	−0.15 (−0.78,0.48)	−0.08 (−0.67,0.50)	**RIS + CBT-F + NBI**

Comparisons between treatments should be read from left to right, and the estimate is in the cell in common between the column-defining treatment and the row-defining treatment. For the primary outcome (attenuated positive psychotic symptoms) estimates, results are SMD (95% CI), where SMD below 0 favors the column-defined treatment. For acceptability, results are OR (95% CI), where OR < 1 favors the row-defined treatment. Significant results are in bold. The order of treatments in the diagonal is arbitrary and does not reflect ranking. ZIP, ziprasidone; NBI, needs-based interventions (including placebo); Om3, omega-3 fatty acids; FFT, family-focused therapy; ARI, aripiprazole; Dser, D-serine; CBT-F, cognitive behavioral therapy (French & Morrison protocol); RIS, risperidone.

Comparison.

Efficacy (attenuated psychotic symptoms; SMD [95% CI]).

Acceptability (dropout; OR [95% CI]).

TABLE 4 | Network meta-analytic relative treatment effects for efficacy and acceptability at 12 months.

OLA + NBI	2.31 (0.64,8.32)	**3.73 (1.01,13.81)**	2.39 (0.73,7.83)	1.98 (0.47,8.32)	2.31 (0.75,7.05)	1.38 (0.30,6.38)
−0.23 (−1.12,0.65)	**Om3 + NBI**	1.61 (0.64,4.07)	1.03 (0.50,2.12)	0.86 (0.29,2.53)	1.00 (0.53,1.86)	0.59 (0.18,2.02)
−0.31 (−1.24,0.63)	−0.07 (−0.80,0.66)	**ARI + NBI**	0.64 (0.32,1.26)	0.53 (0.18,1.57)	0.62 (0.31,1.22)	0.37 (0.11,1.29)
−0.38 (−1.19,0.42)	−0.15 (−0.70,0.40)	−0.08 (−0.63,0.47)	**CBT-F + NBI**	0.83 (0.35,2.00)	0.97 (0.64,1.45)	0.58 (0.19,1.78)
−0.49 (−1.39,0.40)	−0.26 (−0.93,0.41)	−0.19 (−0.90,0.53)	−0.11 (−0.62,0.40)	**RIS + CBT-F + NBI**	1.16 (0.47,2.86)	0.69 (0.17,2.76)
−0.53 (−1.28,0.22)	−0.30 (−0.77,0.17)	−0.23 (−0.78,0.33)	−0.15 (−0.43,0.13)	−0.04 (−0.52,0.44)	**NBI**	0.60 (0.21,1.71)
−0.73 (−1.72,0.26)	−0.50 (−1.30,0.30)	−0.42 (−1.28,0.43)	−0.35 (−1.05,0.36)	−0.24 (−1.04,0.57)	−0.20 (−0.84,0.45)	**IPI**

Comparisons between treatments should be read from left to right, and the estimate is in the cell in common between the column-defining treatment and the row-defining treatment. For the primary outcome (attenuated positive psychotic symptoms) estimates, results are SMD (95% CI), where SMD below 0 favors the column-defined treatment. For acceptability, results are OR (95% CI), where OR < 1 favors the row-defined treatment. Significant results are in bold. The order of treatments in the diagonal is arbitrary and does not reflect ranking. OLA, olanzapine; NBI, needs-based interventions (including placebo); Om3, omega-3 fatty acids; ARI, aripiprazole; CBT-F, cognitive behavioral therapy (French & Morrison protocol); RIS, risperidone; IPI, integrated psychological interventions.

Comparison.

Efficacy (attenuated psychotic symptoms; SMD [95% CI]).

Acceptability (dropout; OR [95% CI]).

3% at 2 years) (9). In a subsequent prospective cohort study, we confirmed that CHR-P individuals meeting the short-lived psychotic episode subgroup criteria have a very high risk of developing persistent psychotic episodes (85). Additional ongoing analyses revealed that these three subgroups are associated with different clinical needs and use of mental health services. These results led us to propose clinical stratification of the CHR-P population across different subgroups (1), which has been endorsed by other leading researchers in this area (2, 86). However, because most trials were conducted before such knowledge emerged, response to preventive treatment was not stratified across these different subgroups and we have been unable to control for this variable in meta-regression analyses. The clinical heterogeneity of this population is further amplified by the heterogeneous accumulation of risk factors for psychosis (5), which is reflected in a variable enrichment

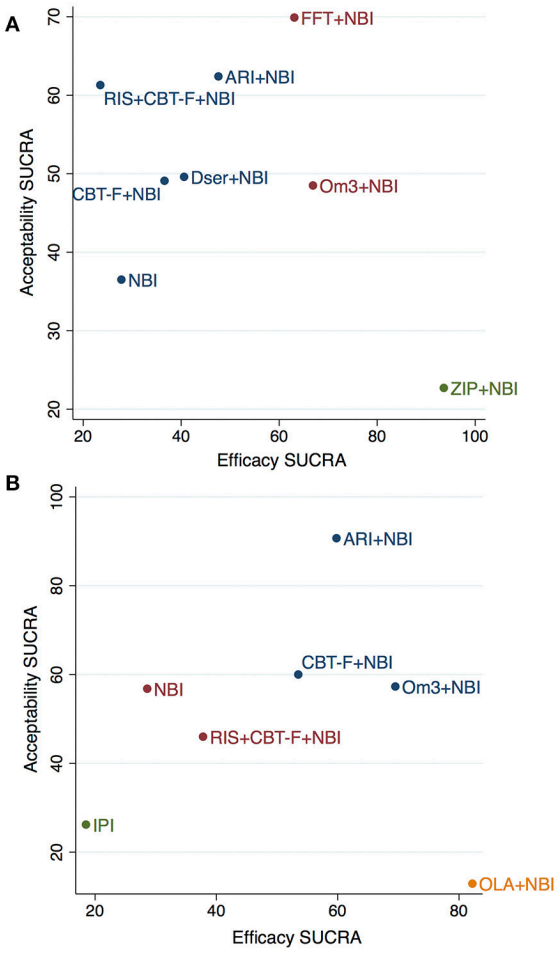

FIGURE 4 | Cluster-ranking of treatments according to SUCRA values for efficacy and acceptability at 6 **(A)** and 12 **(B)** months. Colors reflect treatments belonging to the same cluster. Please note that although the treatments have been ranked, there is no statistical difference between them -with the exception of ziprasidone+NBI for efficacy- (see **Tables 3**,**4** for further details). ARI, aripiprazole; NBI, needs-based interventions (including placebo); CBT-F, cognitive behavioral therapy (French and Morrison protocol); Dser, D-serine; FFT, family-focused therapy; Om3, omega-3 fatty acids; RIS, risperidone; ZIP, ziprasidone; IPI, integrated psychological interventions; OLA, olanzapine.

of risk to psychosis (17) and different clinical outcomes. The latter may include the development of psychosis, persistence of symptoms and comorbidities, or recovery (32). Overall, the above considerations indicate that the "one-size-fits-all" approach to offering preventative strategies to CHR-P individuals is unlikely to work, namely due to the heterogeneity of the CHR-P state. This raises the possibility that the available treatments have been ineffective because they were applied to all CHR-P subjects, rather than to stratified subgroups. For example, a true preventive effect may be difficult to detect in those at low risk or in those who are responding to placebo or low-level needs-based interventions.

These findings may be informative for future research. For example, they suggest that a stratified precision medicine approach may improve the apparent effectiveness of available treatments. Identifying specific factors that predict response to preventive treatments at the individual subject level may substantially advance clinical care for CHR-P individuals by personalizing their preventive interventions. This could be achieved using the existing RCT data under an individual participant data network meta-analytic approach. These advanced meta-analytical approaches allow the stratification of treatment response through the development of predictive risk estimation tools (87) and could potentially produce a breakthrough advancement of clinical knowledge in this area. Our research group has recently completed the protocol for an individual participant data network meta-analyses (PROSPERO 2018 CRD42018089161) which is due to start imminently. At the same time, the lack of convincing evidence for effective treatments should foster refreshed collaborative efforts to test innovative novel treatments for CHR-P individuals. It is important to note that challenges in developing effective preventive treatments are not specific to the CHR-P field but are common across other branches of clinical medicine, such as in the prevention of dementia. Promising compounds are on the horizon. For example, the first ever industry-funded RCT for CHR-P individuals will be investigating the efficacy of a phosphodiesterase inhibitor to prevent psychosis (88). Of relevance, to partially reduce the clinical heterogeneity discussed above, this RCT will focus only on CHR-P individuals presenting with attenuated positive psychotic symptoms and who are enriched in risk as determined by a specific risk stratification algorithm (89). Another promising candidate treatment is cannabidiol, which was found to be well tolerated and reduced symptoms in an early-phase trial in CHR-P individuals, although the full report is not yet available (90, 91). A larger-scale RCT of cannabidiol is due to start at our institute in the near future. The discovery and development of more effective treatments for attenuated positive psychotic symptoms also requires an improved regulatory platform to reliably sustain the next generation of research. For example, while the DSM-5 includes a newly introduced diagnostic category for attenuated psychosis syndrome (92), there will be no similar diagnostic category in the ICD-11. Diagnostic controversies, as well as different methods of ascertainment of attenuated psychotic symptoms [for a comparative analysis of different CHR-P instruments see (34)] are unlikely to facilitate the large-scale collaborations that are necessary to overcome the current limitations.

There are some important limitations to our work. First, the interpretation of negative findings is always challenging. In fact, as noted by leading authors, absence of evidence is not evidence of absence (93). Such an observation is particularly relevant in the case of large CIs, such as those that have been observed in the current analyses (see **Tables 3**, **4**). Therefore, some sizeable effects may still have been missed by our analyses. Furthermore, only 14 RCTs were included, reflecting the scarcity of studies available in this field. Although network meta-analyses are characterized by increased power and precision (94), the geometry of the networks in the current study limited our ability to test for inconsistency, and potentially resulted in more

imprecise estimates and wide 95% CIs. An additional limitation is that the overall quality of our network meta-analysis is dependent on the quality of each included study, most of which were at high or unclear risk of bias. We partially controlled for this problem through assessment of biases and sensitivity analyses. The final limitation concerns the use of dropout for any reason as a proxy measure for acceptability. While this measure is generally accepted in network meta-analyses of RCTs (56–58), it is a rather crude and spurious outcome measure. The use of a more specific side effect outcome could have revealed more subtle differences in acceptability across the available treatments. We have been unable to analyse any specific side effects because these were infrequently reported in the available literature.

CONCLUSIONS

In conclusion, on the basis of the most comprehensive evidence synthesis to date, there is currently no robust evidence to favor specific interventions for improving attenuated positive psychotic symptoms in CHR-P individuals.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data. PF-P designed the study; AC optimized the study; CD and UP conducted the literature search and data extraction; JR extracted the digital data; CD and PF-P conducted the analyses under the supervision of AC and DS; CD and PF-P wrote the first draft of the manuscript; PM reviewed the draft of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00187/full#supplementary-material

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3.2. PART 1 INTERIM SUMMARY

To conclude Part 1 of this thesis, the studies presented in Papers 1 and 2 have demonstrated a lack of evidence for any of the previously tested treatments having superior efficacy relative to any others (Davies *et al*, 2018a, 2018b). These results advance knowledge by challenging the prevailing view that CBT is more efficacious than other currently available treatment options (NICE, 2014). Given that both network meta-analyses included "needs-based interventions", which can be considered the lowest level and nonspecific intervention, the current favouring of CBT over all other treatments does not appear to be supported by evidence. However, absence of evidence is not evidence of absence (Altman and Bland, 1995) and a number of potential methodological factors that may be driving these findings have been discussed, such as the declining transition risks observed in the latest studies, which in turn may lead to underpowered and negative (non-significant) findings. Nevertheless, the lack of significantly superior efficacy in terms of attenuated psychotic symptoms cannot be secondary to low statistical power, which may suggest, rather, that current treatments lack efficacy.

As in psychiatry more broadly, what is being increasingly recognised is that there is unlikely to be one unifying pathophysiological model accounting for the onset of psychosis in all individuals (Millan *et al*, 2016; Paulus and Thompson, 2019). Rather, different CHR-P individuals likely accumulate heterogeneous risk factors for psychosis (Fusar-Poli *et al*, 2017b; Radua *et al*, 2018) which alters their neurobiology in similarly heterogenous ways, but each constellation of pathophysiological processes (in true positives) ultimately ends in the presentation of psychosis—potentially by converging on one or more final common pathways. This represents the "many-to-one" and "one-to-many" mapping issue (Paulus and Thompson, 2019), whereby the brain has many ways of producing the same symptoms, and conversely, similar brain dysfunctions can produce a range of different symptoms (Paulus and Thompson, 2019). It therefore follows that the "one-size-fits-all" approach to CHR-P treatment (and indeed recruitment for clinical trials) is unlikely to work and may be a major factor in the failing of current treatment trials. However, more personalised (even at group level) treatment, targeted to specific pathophysiological processes or neural circuits, may, in future, provide the breakthrough advancement in efficacy the field needs. For example, omega-3 supplementation may prove effective if administered to those who show baseline alterations in membrane fatty acid levels and inflammatory markers (Kane and

Correll, 2017). In future, indicated treatment (and recruitment to specific clinical trials) could be driven by a biologically-informed rationale based on the presence of specific biomarkers. This stratification will require increased understanding of the neurobiological mechanisms underlying psychosis onset within specific strata of individuals (with similar underlying neurobiological alterations). In the meantime, however, and in the absence of full knowledge of the precise pathophysiology, it remains important to find novel (or repurposed) treatments with relevant mechanisms of action based on our current understanding. Part 2 of this thesis aims to address this issue.

PART 2 – POTENTIAL NOVEL TREATMENTS FOR THE CHR-P STATE

4. INTRODUCTION

4.1. POTENTIAL NOVEL COMPOUNDS

As discussed in **Part 1** of this thesis, the lack of apparent efficacy of current interventions indicates that treatments with novel mechanisms of action are needed. Experimental medicine methods, by which a potential candidate compound is tested *in vivo* against a biomarker or neurobiological target associated with the disease of interest, have become a key approach for examining the potential therapeutic benefits of a drug. This can be achieved using placebo-controlled pharmacological challenge and neuroimaging in CHR-P individuals. The success (or failure) of compounds to engage their targets at this proof-of-concept stage can provide invaluable support for early go/no-go decisions for later-phase clinical trials. At the time when the studies within this thesis were conceived, there were a number of potential therapeutics that could have been investigated using these methods. The main possibilities were cannabidiol, omega-3, antidepressants, and oxytocin, which will be briefly reviewed before two experiments using a novel candidate compound are presented.

4.1.1. CANNABIDIOL

Cannabidiol is a major constituent of *cannabis sativa*. In contrast to the psychoactive (and psychotogenic) cannabinoid Δ9-tetrahydrocannabinol (THC) (D'Souza *et al*, 2005), the non-psychoactive compound cannabidiol shows anxiolytic and potential antipsychotic properties (Zuardi *et al*, 2012). Findings that administration of cannabidiol reduced psychotic symptoms in patients with established psychosis (Leweke *et al*, 2012; McGuire *et al*, 2018) has led to the suggestion that it may also have therapeutic potential in those at CHR-P. Interest in cannabidiol is enhanced by its unique (albeit not fully understood) mechanism of action compared to established antipsychotics, as well as its distinct lack of serious adverse effects (Zuardi *et al*, 2012). However, at the time when the experiments in this thesis were designed, an RCT using cannabidiol and magnetic resonance imaging (MRI) in CHR-P individuals was already underway at our institute. This study recently reported that, in 33 CHR-P individuals and 19 controls performing a verbal learning fMRI task, cannabidiol normalised activation in brain regions found to be altered in those at CHR-P under placebo conditions (such as the striatum, medial temporal cortex and midbrain)—regions that are strongly implicated in the onset of psychosis (Bhattacharyya *et al*, 2018). These

early results support the view that cannabidiol may be an effective treatment strategy. A large-scale, multi-site RCT to assess efficacy is now underway.

4.1.2. OMEGA-3

Similarly, when these experiments were first conceived, there was published evidence suggesting promising efficacy of omega-3 supplementation for preventing transition to psychosis (Amminger *et al*, 2010). However, two very large replication trials were already underway at other research centres (Cadenhead *et al*, 2017; McGorry *et al*, 2017) and thus a smaller-scale omega-3 study was not considered the most novel option.

4.1.3. ANTIDEPRESSANTS

As reviewed in Part 1 of this thesis, no RCTs of antidepressants have yet been conducted but a number of naturalistic studies suggest that they are acceptable, well tolerated and tend to be associated with reduced transition relative to antipsychotics (albeit in the context of non-random assignment, antipsychotic nonadherence and likely confounders) (Cornblatt *et al*, 2007). Given that depression and stress have been associated with psychotic symptoms, if antidepressants improve mood, reduce negative appraisals and alter individuals' experience of—and response to—psychosocial stress, then this might be one way in which antidepressants improve CHR-P outcomes (Fusar-Poli *et al*, 2007). An ongoing trial is now testing the potential efficacy of antidepressant interventions (fluoxetine) in CHR-P populations (Nelson *et al*, 2018b; STEP, 2018).

4.1.4. OXYTOCIN

Oxytocin is a hormone and neuropeptide involved in the regulation of social, emotional and autonomic functions, and has been the subject of intense investigation for the potential treatment of numerous neuropsychiatric disorders characterised by social or emotional impairments, such as autism spectrum disorder and schizophrenia (Meyer-Lindenberg *et al*, 2011). Oxytocin can be delivered intranasally and a large body of evidence suggests that it has neurophysiological (Wigton *et al*, 2015), anxiolytic (Neumann and Slattery, 2015), prosocial (Domes *et al*, 2007) and potential antipsychotic properties (Feifel *et al*, 2010). Positive findings from the first studies in established psychosis (Pedersen *et al*, 2011) naturally led to the suggestion that it may be a promising strategy in those at CHR-P. In addition, due to its peripheral roles in birth and lactation, it is already licensed and used routinely in the NHS (NICE, 2008).

4.1.5. INTERIM SUMMARY

Taking the available options together, oxytocin was chosen as the candidate compound for experimental investigation in CHR-P individuals. The decision was based on the following factors: (a) first synthesised in 1952 and as a medicine currently used in the NHS, we have extensive data on its adverse effects, safety and tolerability—repurposing into another branch of medicine would therefore be streamlined (and benign side effect profiles are themselves of critical importance in CHR-P groups); (b) relatedly, by virtue of repurposing a licensed treatment, if it were found to be promising then there would be quicker time-to-translation, lower associated costs and reduced regulatory barriers; (c) oxytocin has a fundamentally different mechanism of action to any of the other options currently available, and to any treatment currently used in CHR-P individuals—while this is also true of cannabidiol and omega-3, oxytocin was the only option not being investigated elsewhere; (d) our institute has long-standing expertise in investigating the neurophysiological effects of oxytocin in the healthy human brain (Paloyelis *et al*, 2016); and (e) furthermore, oxytocin is currently being studied in relation to a number of neuropsychiatric disorders; a better understanding of the neurophysiological basis for its effects in CHR-P individuals may also benefit mental health treatment for other patient populations.

4.2. THE OXYTOCINERGIC SYSTEM

4.2.1. WHAT IS OXYTOCIN?

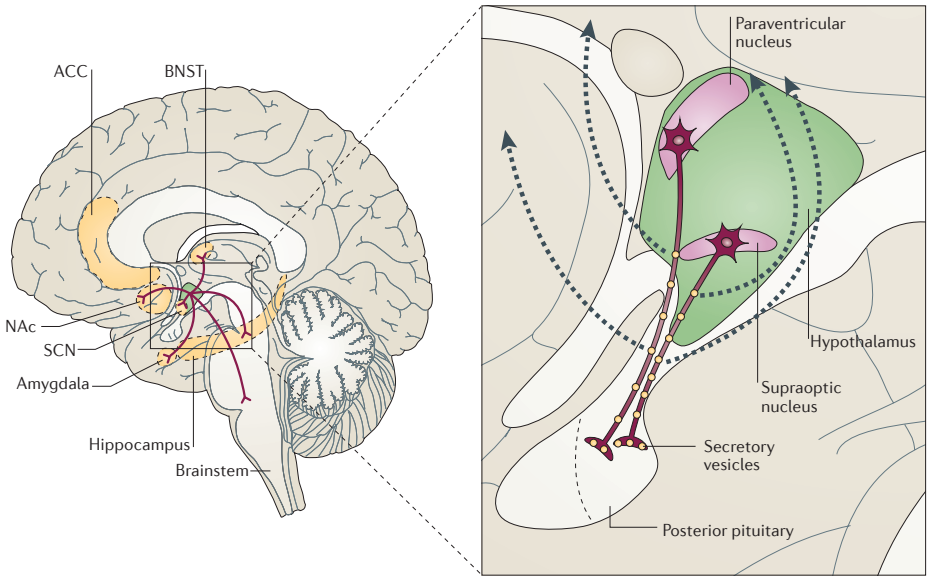
Oxytocin, a highly evolutionarily conserved neuropeptide and hormone, is well known for its role in birth and lactation. However, it also has central effects; it is a robust inhibitor of the hypothalamic–pituitary–adrenal (HPA) axis (Stoop, 2012; Windle *et al*, 2004) and is a key neuromodulator of complex social cognition and behaviours (Meyer-Lindenberg *et al*, 2011). For example, in humans, research has implicated oxytocin in emotion recognition, social memory, social reward, theory of mind (or ‘mentalising’), empathy, trust, autonomic fight or flight response, fear and social stress (Zink and Meyer-Lindenberg, 2012).

4.2.2. SYNTHESIS & RELEASE

Oxytocin is synthesised in magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus (**Figure 4–1**) (Meyer-Lindenberg *et al*, 2011). Following synthesis, oxytocin is stored in large dense-core vesicles which can be secreted from any part of a neuron, including the axon, soma and dendrites (into the

extracellular space/within the blood-brain barrier for central release), or from axon terminals in the posterior pituitary (which lie outside the blood-brain barrier for peripheral release) or terminals in other forebrain/midbrain regions (Johnson and Young, 2017; Knobloch *et al*, 2012; Stoop, 2012). Oxytocin is also produced by parvocellular neurons within the PVN, which have direct axonal projections to numerous brain regions (e.g. amygdala, hippocampus, striatum, bed nucleus of stria terminalis and brainstem (**Figure 4–1**) (Knobloch *et al*, 2012; Meyer-Lindenberg *et al*, 2011)), although these data are not available specifically for humans (Stoop, 2012). Thus, oxytocin delivery is thought to occur in a multimodal manner via two modes of release: (i) slow "unwired" dendritic secretion into the local hypothalamic environment with passive diffusion to global/distal regions (spread neuromodulation), and (ii) faster "wired" precise axonal release to targeted brain regions expressing oxytocin receptors (point-to-point transmission) (Landgraf and Neumann, 2004; Stoop, 2012).

Figure 4–1. Neurophysiology of oxytocin and vasopressin.



Reprinted by permission from Springer Nature © 2011: Nature Reviews Neuroscience (Meyer-Lindenberg *et al*, 2011). ACC, anterior cingulate cortex; BNST, bed nucleus of the stria terminalis; NAc, nucleus accumbens; SCN, suprachiasmatic nucleus.

4.2.3. RECEPTOR DISTRIBUTION

Oxytocin receptors have been detected in many cortical/subcortical brain regions in animals which are relevant to psychosis pathophysiology (e.g. hippocampus, amygdala, thalamus, neocortex) (Loup *et al*, 1991). However, significant species-specific differences have limited direct translation to humans and thus precise receptor

distributions remain mostly unclear. However, recent work on the distribution of oxytocin receptor gene (OXTR) and CD38 (a gene involved in oxytocin secretion) messenger ribonucleic acid (mRNA) expression suggests that humans do have similar receptor distributions (Quintana *et al*, 2019a). Compared to average brain expression, oxytocin-pathway gene expression was found to be enriched in the olfactory region, caudate, putamen, pallidum, thalamus, hippocampus, parahippocampal cortex and amygdala, and decreased expression was found in the cerebellum (Quintana *et al*, 2019a), although not all findings survived multiplicity correction. It is worth noting that oxytocin can also bind vasopressin receptors (of which there are three) but does so with approximately 100-fold weaker affinity (Manning *et al*, 2012). Until recently, there was no selective radiotracer that could penetrate the blood-brain barrier for imaging the oxytocinergic system in humans *in vivo* (Smith *et al*, 2012). Early studies using autoradiography in human postmortem tissue showed radiotracer receptor binding in the brainstem (Freeman *et al*, 2016), amygdala, anterior cingulate, hypothalamus (Boccia *et al*, 2013), basal nucleus of Meynert, globus pallidus, ventral pallidum and septal nuclei (Loup *et al*, 1991). However, due to the high degree of cross-affinity for oxytocin and vasopressin receptors, these methods are not considered sufficiently selective and the differing methods used (i.e. various antibodies or ligands) have given rise to conflicting results. Recently, a novel oxytocin receptor tracer has been developed for probing nose-to-brain transport uptake *in vivo* (Beard *et al*, 2018). A significant signal was found in the olfactory bulb (implying direct nose-to-brain transport via nerve fibres) but the tracer was not able to penetrate deeper brain tissue (Beard *et al*, 2018). Results of further research in this field are awaited.

4.2.4. RECEPTOR FUNCTION

Although only one ‘type’ of oxytocin receptor exists, binding of oxytocin to its receptor (which is G-protein coupled) can initiate one of a number of complex intracellular cascades, mediated by the coupling of different G proteins—thought to underlie its differential functional effects in different brain regions (for detailed review, see (Stoop, 2012)). Preclinical work suggests that the majority of oxytocin receptors are located on GABAergic interneurons (or poised at GABAergic synapses) to control inhibitory transmission (Marlin *et al*, 2015; Mitre *et al*, 2016). The inhibitory effects of oxytocin binding are thought to be caused by enhanced excitability of amygdala (Huber *et al*, 2005; Knobloch *et al*, 2012), hippocampal (Harden and Frazier, 2016; Owen *et al*, 2013; Raam *et al*, 2017) and prefrontal cortical (Nakajima *et al*, 2014) GABAergic interneurons (likely amongst other regions), leading to increased GABA release and

therefore inhibition. In the rat amygdala, for example, administration of oxytocin is associated with analogous effects to benzodiazepines (although they act on different components of the circuit) and when co-administered, oxytocin potentiates the effects of benzodiazepines such that lower doses can be used (Viviani *et al*, 2010). Other work suggests that oxytocin suppresses glutamatergic neurotransmission in infralimbic cortex by decreasing presynaptic glutamate release via a cannabinoid-1 receptor dependent mechanism (Ninan, 2011). Oxytocin receptors are also expressed on excitatory glutamatergic cells (Raam *et al*, 2017) and serotonergic neurons (Dölen *et al*, 2013; Yoshida *et al*, 2009).

4.2.5. PHARMACOKINETICS/ELIMINATION HALF-LIFE

Centrally-released endogenous oxytocin is metabolised by aminopeptidases in brain tissue and any remainder is cleared via flow into subarachnoid space and/or transport into blood (Stoop, 2012). In humans, the elimination half-life of oxytocin in cerebrospinal fluid (CSF) is relatively long (compared to classical neurotransmitters) at ~20 minutes, 2–3 mins in plasma and ~1 minute in brain (Ludwig and Leng, 2006). In mostly small, initial studies, intranasally delivered oxytocin was found to increase plasma concentrations (Burri *et al*, 2008) with peaks at 10–40 minutes post-administration (Gossen *et al*, 2012; Landgraf, 1985; Striepens *et al*, 2013), but a recent study reported significant peaks in the CSF beginning only after 75 minutes (Striepens *et al*, 2013).

4.2.6. VASOPRESSIN

It is worth noting that arginine vasopressin, the cousin nonapeptide of oxytocin, is generally thought to have diametrically opposing physiological effects to oxytocin (Neumann and Landgraf, 2012). Vasopressin has similar binding affinities for oxytocin and vasopressin receptors, but oxytocin has 100-fold higher affinity for oxytocin (vs vasopressin) receptors (Stoop, 2012). Despite cross-reactivity of oxytocin with vasopressin (particularly 1a) receptors, receptor distributions differ markedly (Stoop, 2012) and it is thought that the distribution patterns allow for social cognitive and behavioural states to be precisely balanced by the coordinated release of the two peptides (with distinct spatial distributions) (Landgraf and Neumann, 2004; Neumann and Landgraf, 2012). For example, in the amygdala, vasopressin and oxytocin receptors are differentially expressed in different subnuclei (which subserve different functions),

enabling the precise modulation of different behavioural phenomena (i.e. balance of approach/avoidance behaviour) (Huber *et al*, 2005).

Importantly, the cross-reactivity of oxytocin is a critical factor for consideration in studies using intranasal oxytocin as a candidate therapy—if not enough oxytocin is delivered then there may be no observed effects, but if too much is given (with oxytocin saturating oxytocin receptors and now binding vasopressin receptors), the measurable effect of oxytocin at its receptor may be cancelled out by the opposing physiological effects of oxytocin binding to vasopressin receptors (Galbusera *et al*, 2017).

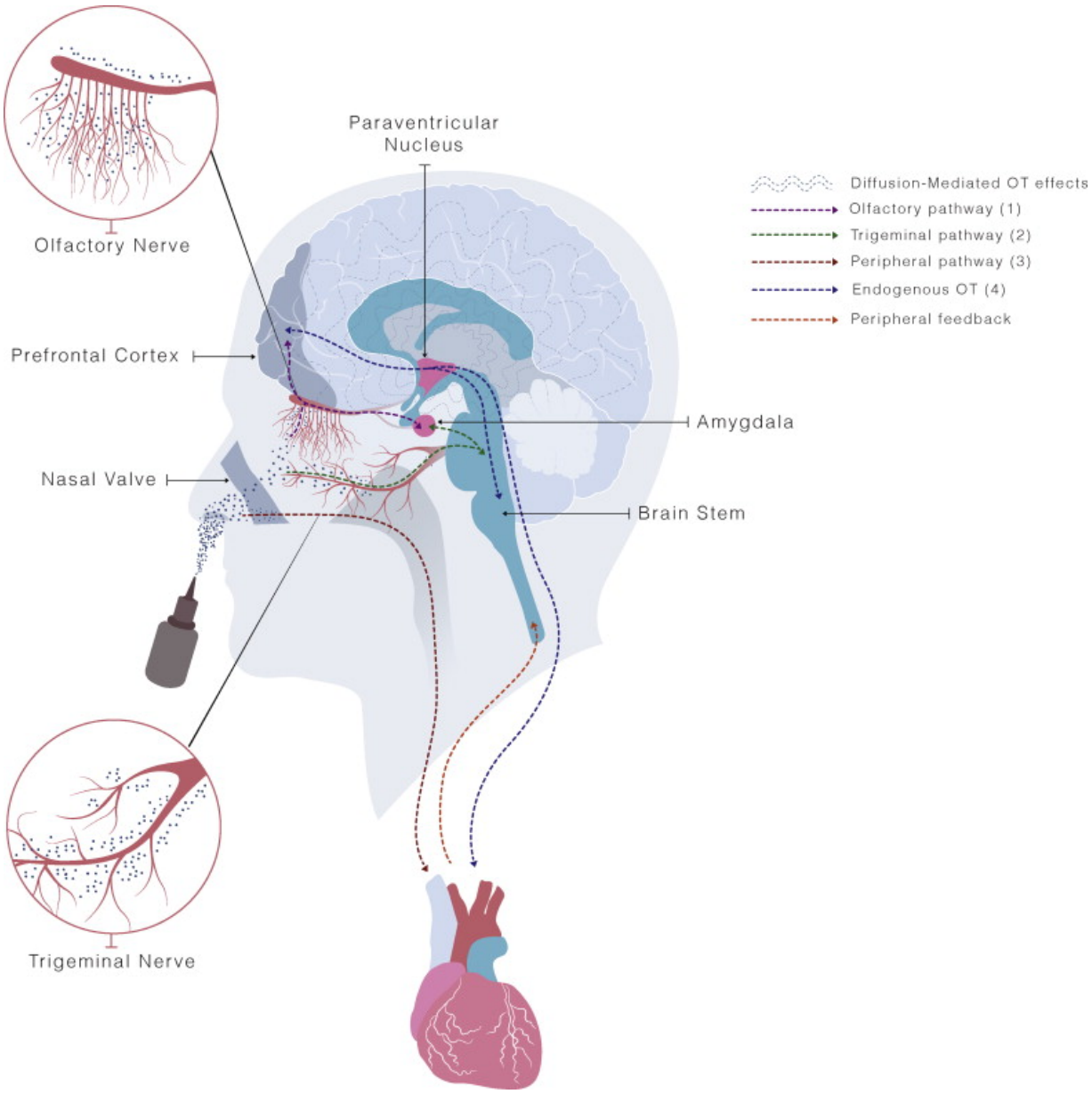
4.2.7. NOSE-TO-BRAIN TRANSPORT OF INTRANASAL OXYTOCIN

Intranasal oxytocin is thought to reach the brain via a number of potential pathways, including (1) the olfactory pathway, and (2) the trigeminal pathway, in addition to systemic circulation via (3) the peripheral pathway (**Figure 4–2** below; for review see (Quintana *et al*, 2018)). Some authors have questioned whether such nose-to-brain transport exists and suggest that any central effects are secondary to large increases in peripheral oxytocin levels that then either cross the blood-brain barrier or act on the gastrointestinal tract, heart or reproductive system (Leng and Ludwig, 2016). If this is the case, then studies that compare intranasally delivered oxytocin to peripherally administrated (i.e. intravenous) oxytocin should produce the same profile of effects. A number of studies have now tested this notion in rodents (Galbusera *et al*, 2017; Williams *et al*, 1994), primates and humans (Martins *et al*, 2019; Quintana *et al*, 2016, 2017), and there is converging evidence that peripheral doses do not recapitulate the full central (neural) effects of intranasally delivered oxytocin. For example, in order to determine whether intranasal administration represents a privileged route for the central delivery of oxytocin, a recent, well-designed study contrasted two methods of intranasal administration (a standard nasal spray and a nebuliser) to intravenous administration (Martins *et al*, 2019). The authors reported a number of important findings. First, that the oxytocin-induced decreases in cerebral blood flow (CBF) in the amygdala and anterior cingulate cortex (ACC), after both intravenous and nasal spray administration, could be explained by increases in oxytocin plasma concentrations, which suggests that neural effects are not solely derived from direct nose-to-brain transport (Martins *et al*, 2019). However, changes in oxytocin plasma concentrations could not account for any of the other oxytocin-induced changes observed in CBF (with either the nasal spray or nebuliser), providing robust pharmacodynamic evidence consistent with the existence of direct nose-to-brain transport in humans (Martins *et al*, 2019). Finally, compared to

the nasal spray, nebuliser-administered oxytocin (which is thought to improve deposition of oxytocin to nasal regions putatively involved in direct nose-to-brain transport) resulted in markedly different patterns of CBF changes, which the authors suggest may be due to increased amounts of oxytocin reaching the brain (Martins *et al*, 2019). Further discussion of the effects of oxytocin on CBF in humans is presented in section 4.3.4 (on page 83).

Finally, as previously mentioned, a novel oxytocin receptor radiotracer was recently developed for directly probing nose-to-brain transport in humans (Beard *et al*, 2018). A significant signal was found in the olfactory bulb (supporting direct nose-to-brain transport via nerve fibres) but this tracer was not able to penetrate deeper brain tissue (Beard *et al*, 2018). Results from further radiotracer studies are awaited.

Figure 4–2. Intranasal oxytocin pathways and destinations.



Reprinted from (Quintana *et al*, 2015) with permission from Elsevier.

Caption: Oxytocin (OT) can access the following destinations after intranasal administration; the amygdala from deposition on the olfactory epithelium, through the olfactory bulb then via axonal projections as well as diffusion through the cerebrospinal fluid (CSF) (1), brainstem via deposition on the respiratory epithelium trigeminal nerve fibres (2), the periphery via blood capillaries in the olfactory and respiratory epithelia (3). Delivery of OT via olfactory and trigeminal pathways may stimulate the production of endogenous OT from the paraventricular nucleus (PVN), delivering OT to both the prefrontal and brainstem regions, as well as systemic circulation (4).

4.3. OXYTOCIN, PSYCHOSIS & THE CHR-P STATE

In addition to the introductions of Paper 3 (starting on on page 93) and Paper 4 (on page 118), in this section I present a review of previous oxytocin research relating to CHR-P psychopathology and pathophysiology. First, given the lack of research in those specifically at CHR-P, the results of RCTs (which have assessed symptoms) in patients with established psychotic disorders are reviewed. This is followed by evidence linking oxytocin's effects on social, emotional and autonomic functioning with the CHR-P state, and a brief review of related functional magnetic resonance imaging (fMRI) studies. Finally, I conclude this introduction by presenting results from more quantitative MRI methods which allow examination of the neurophysiological effects of oxytocin on indices of neural function strongly linked to psychosis onset.

4.3.1. SYMPTOMS OF PSYCHOSIS

Initially, oxytocin was considered a promising compound for treating both positive and negative symptoms of psychosis (Feifel *et al*, 2015). Numerous studies have now been conducted in patients with established psychotic disorders (mostly schizophrenia) although the results have been decidedly mixed (Bradley and Woolley, 2017). For example, in a crossover trial of adjunctive oxytocin (40IU twice/day) vs placebo (N=15), oxytocin significantly reduced Positive and Negative Syndrome Scale (PANSS) scores (both positive and negative) by the three-week study endpoint (Feifel *et al*, 2010). Another study (N=20) reported improved positive symptoms, paranoia and general psychopathology within the oxytocin group from baseline to follow up after two weeks of 24IU oxytocin (twice/day) (Pedersen *et al*, 2011). One study of 20IU/day oxytocin over 3 weeks found no difference in positive, negative or global symptoms, except for improved negative symptoms in the subset of inpatients (Lee *et al*, 2013). A larger RCT (N=40) titrating to 40IU (twice/day) for 8 weeks found significant effects on positive, negative, general and total PANSS scores (Modabbernia *et al*, 2013). However, in an 8-month crossover RCT of 40IU oxytocin (N=32), there were no effects on positive or negative symptoms, or any other measured outcome (Dagani *et al*, 2016). A recent study of 55 patients (24IU twice/day for 12 weeks) reported no effects on positive, negative, general or total PANSS scores (Jarskog *et al*, 2017), although a significant change-from-baseline improvement in negative symptoms was observed in the oxytocin group. Support for beneficial effects on negative symptoms was found in one further study (Gibson *et al*, 2014), although two others report no effect on positive or negative symptoms (Cacciotti-Saija *et al*, 2015; Davis *et al*, 2014). Such conflicting

results have prompted a number of meta-analyses. While an early meta-analysis concluded that oxytocin was superior to placebo for decreasing general PANSS scores (Oya *et al*, 2016), a more recent and sophisticated Bayesian meta-analysis concluded that oxytocin does not improve any aspect of schizophrenia psychopathology (positive, negative, general or total symptoms) (Williams and Bürkner, 2017).

While the meta-analytic results relating to symptoms are sobering, there are a number of important factors which likely contribute to the negative or inconsistent findings. This includes the fact that all of the aforementioned studies took an 'add-on' approach, using adjunctive oxytocin or placebo in patients already receiving (and usually stable on) antipsychotic medication. Three of the studies investigated oxytocin in addition to social skills training (Cacciotti-Saija *et al*, 2015; Davis *et al*, 2014; Gibson *et al*, 2014). Concomitant antipsychotic treatment may create a 'ceiling' beyond which any further benefits are difficult to observe. Indeed, due to the smaller effect size associated with add-on studies, the required sample sizes are much larger than have been used in practice (Shilling and Feifel, 2016). A related point is that in the absence of monotherapy RCTs (which are unlikely to gain ethical approval for patients with established psychosis), direct estimates of oxytocin's efficacy cannot be derived (Feifel *et al*, 2015). In addition, it has been suggested that antipsychotics interact with or moderate the effects of oxytocin, and given that all trials were add-on studies, this could underlie some of the non-significant findings and high levels of between-study/subject heterogeneity (Bradley and Woolley, 2017). These limitations also underscore the benefits of assessing potential novel treatments in those at CHR-P; most CHR-P individuals are antipsychotic naïve (Fusar-Poli *et al*, 2015b) and monotherapy trials are generally considered acceptable (usually combined with needs-based interventions).

4.3.2. SOCIAL, EMOTIONAL & AUTONOMIC FUNCTIONING

Given the substantial evidence from preclinical studies for oxytocin's role in social and affiliative behaviours (Neumann and Landgraf, 2012; Stoop, 2012), the leap to conceptualising oxytocin as a potential therapy for human disorders characterised by social dysfunction is perhaps not surprising. Impairments of social cognition, which include emotional recognition, theory of mind, social perception and attributional style (Green *et al*, 2008), are a primary cause of disability in psychosis (Fett *et al*, 2011) and respond poorly to current treatments. Emotion dysregulation and social cognition problems are also common in individuals at CHR-P (Fusar-Poli *et al*, 2012b), are a key source of distress and contribute strongly to loss of functioning (Barbato *et al*, 2013;

Cotter *et al*, 2017; Van Donkersgoed *et al*, 2015). Studies also report evidence of HPA axis alterations in CHR-P individuals, including enhanced stress sensitivity/intolerance (DeVylder *et al*, 2012), altered cortisol response (Corcoran *et al*, 2012; Day *et al*, 2014; Walker *et al*, 2010) and high levels of anxiety (McAusland *et al*, 2017) which are associated with later transition to psychosis (Owens *et al*, 2005).

Oxytocin has numerous prosocial, anxiolytic and antipsychotic-like effects in animals (Caldwell *et al*, 2009; Feifel and Reza, 1999; Lee *et al*, 2005) and is a robust inhibitor of the HPA axis (Windle *et al*, 2004). In healthy individuals, offline studies show that oxytocin improves theory of mind performance (Domes *et al*, 2007) whilst decreasing arousal and aversion towards negative or threatening social stimuli (Eckstein *et al*, 2015) (for review see (Zink and Meyer-Lindenberg, 2012)). Oxytocin also improves performance (Domes *et al*, 2007) in the 'Reading the Mind in the Eyes' Test (RMET) (Baron-Cohen *et al*, 2001), a task commonly used in social cognition research to index ability to interpret mental states from social cues from the eye region, and in which CHR-P individuals show impaired functioning (Zhang *et al*, 2016). In patients with psychosis, oxytocin improves emotional processing and social cognition, particularly higher-order social cognition (Brambilla *et al*, 2016; Davis *et al*, 2013, 2014; Guastella *et al*, 2015; Pedersen *et al*, 2011). However, not all studies show significant effects (Halverson *et al*, 2019) and hope of oxytocin's potential for improving social cognition in established psychosis has been somewhat stifled by recent negative meta-analytic findings (Bürkner *et al*, 2017).

How oxytocin brings about these emotional processing and social cognition effects at the neural level is less clear. Oxytocin can increase attention to biologically relevant stimuli (Gordon *et al*, 2016), memory for social stimuli (Guastella *et al*, 2008b; Maroun and Wagner, 2015) and eye-gaze (Auyeung *et al*, 2015; Guastella *et al*, 2008a), seemingly via its modulation of limbic (and particularly amygdala subnuclei) activity (Gamer *et al*, 2010). Eye-gaze is a fundamental form of human communication and amount of eye-gaze is predictive of successful inference of others' intentions and the meaning of social situations (Guastella *et al*, 2008a; Klin *et al*, 2002). These effects offer one mechanism by which oxytocin may improve inference of others' mental state and enhance emotion recognition.

4.3.3. FUNCTIONAL NEUROIMAGING

Despite the markedly mixed findings regarding the effects of oxytocin on symptoms and behaviour, evidence that oxytocin can modulate brain function, as measured using blood-oxygenation-level-dependent (BOLD) response fMRI, is much less ambiguous (Grace *et al*, 2018; Wigton *et al*, 2015; Zink and Meyer-Lindenberg, 2012). Individuals at CHR-P show altered neural responses to various social (and non-social, see below) cognitive stimuli in fMRI studies, particularly in emotion recognition/regulation (Tseng *et al*, 2016; van der Velde *et al*, 2015) and response to threat (Wolf *et al*, 2015). Early fMRI studies demonstrated that the amygdala, a crucial node for social and emotion processing, appears to be a main target for oxytocin-mediated effects (Bethlehem *et al*, 2013; Eckstein *et al*, 2015; Kirsch, 2005). Impaired processing of emotional stimuli in early and established psychosis has been associated with amygdala dysfunction (Anticevic *et al*, 2012; Modinos *et al*, 2015b; Pinkham *et al*, 2011; Taylor *et al*, 2012), and altered amygdala connectivity has been observed across the psychosis spectrum (Anticevic *et al*, 2014). Oxytocin robustly modulates amygdala activation (Wigton *et al*, 2015) as well as the functional connectivity of the amygdala to other important nodes of social/emotional cognition and the stress response, such as the precuneus (Kumar *et al*, 2015), anterior cingulate cortex (ACC) (Riem *et al*, 2012), medial prefrontal cortex (Sripada *et al*, 2013) and brainstem (Kirsch, 2005). Many of these areas and other prefrontal, limbic and temporal regions are differentially engaged by CHR-P subjects (Brüne *et al*, 2011; Takano *et al*, 2017) and other high-risk individuals (Marjoram *et al*, 2006; Mohnke *et al*, 2016) during social cognition fMRI tasks. However, whether oxytocin increases or decreases the BOLD signal appears to vary by context, specific fMRI task, brain region, sex of the population under study and potentially many other variables, such as ingroup-outgroup status and oxytocin receptor genotype (Bartz *et al*, 2011; Grace *et al*, 2018; Wigton *et al*, 2015; Zink and Meyer-Lindenberg, 2012).

In addition, resting state functional connectivity between amygdala and prefrontal cortex, and between amygdala and brainstem, is altered in high risk populations (Anticevic *et al*, 2014; Gee *et al*, 2012) and is thought to be a neural correlate of autonomic dysfunction and social-cognitive deficits (Rosenfeld *et al*, 2011). In healthy individuals and those with other disorders characterised by social dysfunction, numerous studies have demonstrated effects of oxytocin on connectivity between various nodes relevant to CHR-P pathophysiology, including the hippocampus, amygdala, striatum, cerebellum, ACC, precuneus and other regions known to be

involved with social and emotional processing (Bethlehem *et al*, 2013; Dodhia *et al*, 2014; Eckstein *et al*, 2017; Gorka *et al*, 2014; Grace *et al*, 2018; Riem *et al*, 2012; Zhao *et al*, 2018).

Oxytocin has also been found to modulate other (non-social) cognitive processes such as spatial and episodic memory and cognitive flexibility (Bradley and Woolley, 2017; Chini *et al*, 2014) and further key targets for oxytocin-mediated fMRI effects include the striatum, midbrain, and hippocampus (Baumgartner *et al*, 2008; Bethlehem *et al*, 2013; Hu *et al*, 2015; Rilling *et al*, 2012, 2014; Zhao *et al*, 2018)—regions critically implicated in the onset of psychosis and the functioning of which are key targets for novel pharmacotherapies (Lieberman *et al*, 2018; Millan *et al*, 2016).

4.3.4. NEUROPHYSIOLOGICAL NEUROIMAGING

The neuroimaging evidence for oxytocin's effects has, until recently, been limited to BOLD fMRI studies. These have been invaluable in showing that oxytocin acutely modulates brain function in key regions linked to psychosis pathophysiology, but they are limited by the need to contrast "active" and "baseline" conditions and so only tell us about relative effects during specific social, emotional or other cognitive tasks. That is, effects of oxytocin in such paradigms are restricted to modulatory effects confined within the neural networks engaged by the task (Galbusera *et al*, 2017).

More recently, the neurophysiological effects of oxytocin have been of increasing interest to researchers. As with fMRI, methods such as Arterial Spin Labelling (ASL) and Proton Magnetic Resonance Spectroscopy (1H-MRS) are more sensitive methods for assessing the effects of drugs than behavioural studies. That is, they can illuminate potential disease target-engaging effects even when there may be no observable difference at the behavioural or symptom level. Another benefit is that both ASL and 1H-MRS provide results of absolute quantitation and tell us about the effects of drugs in the 'resting state' (i.e. in the absence of a specific cognitive task or other manipulation). In the case of ASL, this also allows researchers to look for effects across the brain without being spatially limited to those regions engaged by a specific cognitive fMRI task.

Oxytocin and Arterial Spin Labelling (ASL)

In the absence of Positron Emission Tomography (PET) receptor studies, the spatiotemporal profile of effects of exogenously administered oxytocin in living human

brain has—until recently—been relatively unclear. In rodents, however, a study of cerebral blood volumes showed that intranasal oxytocin rapidly elicits (a) transient activation of numerous cortical regions, (b) sustained activation of hippocampal and forebrain regions, and (c) multi-band power increases in hippocampal local field potential recordings (Galbusera *et al*, 2017). Evidence that oxytocin may have similar regional effects in humans came from a study that mapped the oxytocinergic network using ASL (as a non-invasive pharmacodynamic biomarker) combined with intranasal oxytocin vs placebo in parallel groups of healthy males (Paloyelis *et al*, 2016). Here, they found that oxytocin induced widespread increases in resting cerebral blood flow (rCBF, also termed 'perfusion') in regions that (a) are thought to express oxytocin receptors (Quintana *et al*, 2019a), (b) are known mediators of social and emotional processing (Adolphs, 2003; Meyer-Lindenberg *et al*, 2011), and (c) are key regions implicated in psychosis pathophysiology (Allen *et al*, 2016; Lieberman *et al*, 2018; Millan *et al*, 2016; Smieskova *et al*, 2010). Specifically, in a large left-lateralised subcortical cluster, oxytocin modulated (increased) perfusion in the caudate nucleus, ventral striatum, pallidum, amygdala, hippocampus, hypothalamus and ventral midbrain (Paloyelis *et al*, 2016). Other clusters of increased perfusion included the ACC, large prefrontal and temporal cortical clusters and the cerebellum. This study also reported the temporal dynamics of oxytocin administration, showing sustained effects from 25–78 minutes post-administration, with a peak response at 39–51 minutes followed by a gradual diminution thereafter (Paloyelis *et al*, 2016). In a more recent (within-subject crossover) study, the same authors found that oxytocin *decreased* perfusion in a left-lateralised limbic cluster which included the hippocampus, amygdala and insula, and in clusters spanning the ACC, frontal cortex, brainstem and cerebellum; increased perfusion was observed in further frontal, parietal and temporal regions (Martins *et al*, 2019).

The subcortical findings are of particular interest to CHR-P research because accumulating evidence suggests that altered cerebral perfusion in these regions is central to the development of psychosis, and compounds that can modulate perfusion (particularly in the hippocampus) have been suggested as promising therapeutic strategies (Lieberman *et al*, 2018). In fact, one of the only neuroimaging findings to have been replicated in CHR-P groups to date is that hippocampal perfusion is increased in CHR-P individuals relative to controls, and in those with poor vs good clinical outcomes (Allen *et al*, 2016, 2018; Schobel *et al*, 2013) (for full discussion see Introduction, **Paper 3** – Oxytocin ASL, starting on page 93). Research is also starting

to report altered perfusion in additional regions. For example, relative to healthy controls, CHR-P individuals show reduced prefrontal but increased striatal blood flow, with striatal blood flow significantly (positively) associated with levels of attenuated psychotic symptoms (Kindler *et al*, 2018).

While the known effects of oxytocin on cerebral blood flow appear directly relevant to the therapeutic targets indicated in those at CHR-P, no study has tested the effects of oxytocin on cerebral perfusion in this patient population. Such an investigation would elucidate potential therapeutic properties of oxytocin and, if successful, would provide proof-of-concept evidence for larger clinical trials in this patient group.

Oxytocin and Proton Magnetic Resonance Spectroscopy (1H-MRS)

Another component of the most established models of psychosis onset is that of disturbed neurochemical metabolites, particularly the neurotransmitter glutamate. Accumulating evidence suggests that the pathophysiological processes underlying psychosis onset may be driven by dysregulated glutamate neurotransmission (Lieberman *et al*, 2018), with hypofunction of N-methyl-D-aspartate receptors (NMDAR) on γ-aminobutyric acid (GABA)-ergic interneurons leading to disinhibition of pyramidal cells, and via polysynaptic projection pathways from the hippocampus to the midbrain/striatum, to midbrain hyperdopaminergia (Lisman *et al*, 2008; Modinos *et al*, 2015a). In animals, oxytocin has been found to modulate this neural circuit by enhancing the signal-to-noise ratio of pyramidal cell firing via the targeting of GABAergic interneuron function (Owen *et al*, 2013; Zaninetti and Raggenbass, 2000)—dysfunction of which are strongly implicated in psychosis onset (Lieberman *et al*, 2018; Lisman *et al*, 2008; Modinos *et al*, 2015a).

Evidence for neurochemical perturbations in CHR-P individuals—and the potentially relevant properties of oxytocin—are reviewed in full in Paper 4 (see Introduction, **Paper 4** – Oxytocin 1H-MRS, starting on page 118). However, in brief, numerous studies have reported alterations in glutamate (or glutamate plus glutamine; Glx) levels in people at high risk of psychosis vs controls, particularly in the hippocampus (Bloemen *et al*, 2011; Shakory *et al*, 2018), frontal cortex (Merritt *et al*, 2016), ACC (Stone *et al*, 2009; Tibbo *et al*, 2004) and thalamus (Stone *et al*, 2009). Within CHR-P samples, baseline hippocampal glutamate is elevated in those who transition (vs those who do not) and in those with poor (vs good) functional outcomes (Bossong *et al*, 2019). Novel pharmacotherapies that can modulate the glutamate system—or

associated neural circuits—have therefore become a target for drug development (Lieberman *et al*, 2018; Millan *et al*, 2016). Whether oxytocin can modulate metabolite concentrations in CHR-P individuals is unknown.

4.4. RATIONALE & EXPERIMENTAL METHODS

4.4.1. TREATMENT TARGETS & STUDY DESIGN

Converging evidence suggests that hippocampal and neurochemical dysfunction plays a key role in the onset of psychosis, and novel compounds that can modulate hippocampal perfusion and glutamatergic function are of significant interest to the CHR-P field. As discussed in full in Paper 3, altered hippocampal perfusion is among the few MRI findings to have been replicated in CHR-P groups, and as a proposed driver of psychosis onset, represents a disease-modifying target. While animal and initial human work suggest that oxytocin may modulate hippocampal function and neurochemistry in those at CHR-P, this has never been tested before.

In order to investigate the neurophysiological effects of oxytocin in people at CHR-P, in the experiments that follow in **Paper 3** and **Paper 4**, I compare the effects of oxytocin vs placebo on (a) hippocampal perfusion using ASL, and (b) neurochemical metabolites using 1H-MRS, in a double-blind, placebo-controlled, crossover MRI study. As discussed in section 4.3.4, these two MRI modalities were selected based on their ability to measure two key indices of neural dysfunction implicated in the onset of psychosis. If oxytocin demonstrates an ability to alter hippocampal perfusion or neurochemical metabolite levels, this would provide proof-of-concept evidence of disease-target engagement for larger clinical trials. This methodological approach (termed experimental medicine), whereby one tests the ability of a compound to engage specific (often intermediate) targets linked to disease (such as hippocampal perfusion), has specifically been recommended for use with oxytocin and CHR-P research (Bradley and Woolley, 2017; Lieberman *et al*, 2018). While the full background and methods are reported in **Paper 3** and **Paper 4**, an overview of the two experimental techniques and a general power calculation are presented below.

4.4.2. POWER CALCULATION

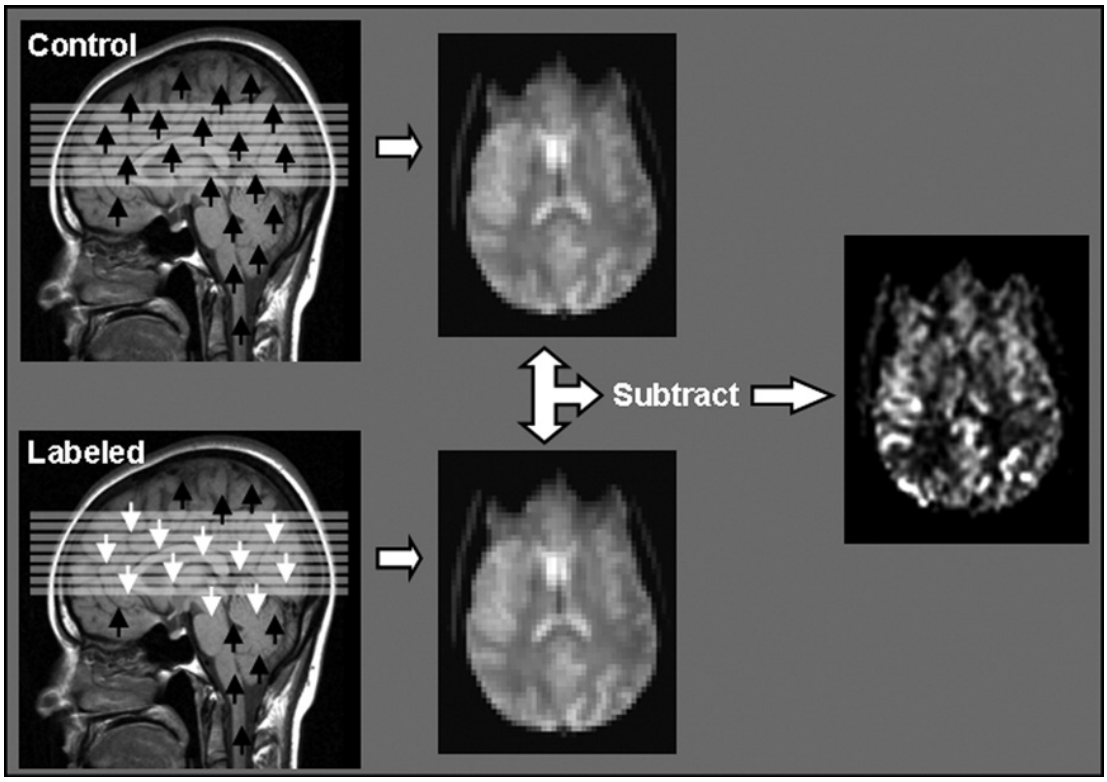
The primary outcome was the within-subject effect of oxytocin vs placebo on hippocampal perfusion. At the time of designing the study, there was no direct effect size (for effects of oxytocin on perfusion in humans) from previous studies on which to base our power calculations. However, a meta-analysis of the neurophysiological

effects of oxytocin in controls performing social cognition fMRI tasks reported combined effect sizes (d) of 0.82 and 1.56 (large effects) in the amygdala and temporal lobes, respectively (Wigton *et al*, 2015). A power calculation using G*Power3 indicated that 30 subjects in a matched-pairs design was sufficient to detect an effect size (d_z) of 0.53 (medium effect size), for α=0.05 (two-tailed) and β=0.20 (power of 80%).

4.4.3. ARTERIAL SPIN LABELLING – METHODS

ASL is an MRI technique for measuring cerebral perfusion (cerebral blood flow, CBF) in living human brain (Grade *et al*, 2015). The technique uses the water protons in arterial blood as an intrinsic tracer, thereby omitting the need for an exogenous contrast agent (such as gadolinium, which is used in dynamic susceptibility contrast perfusion imaging) and is thus non-invasive (Alsop *et al*, 2015). In contrast to fMRI, this technique gives results of absolute quantitation; the difference in signal between the tagged and untagged images (see below) represents blood flow in a specific voxel/region across a specific unit of time. The units are therefore given in millilitres of blood per 100g of tissue per minute (ml/min/100g). As depicted in **Figure 4–3**, specific "slabs" or planes of arterial blood, selected by gradient magnetic fields, are "tagged" (or "labelled") by the application of a 180-degree radiofrequency (inversion) pulse as they travel through the neck towards the brain. This causes the protons in the tagged blood to invert 180 degrees (i.e. the longitudinal magnetisation [T1] is inverted). After a specific period of time (called the post-labelling delay), the signal is measured. This gives time for the labelled blood from the neck to reach the brain slice of interest. Displacement of the original blood (where the spins were fully relaxed, i.e. there was static tissue magnetisation) with the labelled blood (with its inverted spins) causes a reduction in the longitudinal magnetisation, which creates a measurable signal. After collection of this labelled image, the procedure is repeated but without the initial inversion of the magnetisation, to acquire an unlabelled (control) image. Subtraction of the labelled from the unlabelled image results in a difference image (the perfusion-weighted image), where each voxel has an associated quantitative value representing perfusion in that voxel. In pharmacological challenge studies, two perfusion images, one from each drug condition, can then be statistically compared to find the absolute difference in cerebral perfusion caused by the experimental manipulation (i.e. oxytocin vs placebo).

Figure 4–3. Arterial Spin Labelling (ASL) perfusion MRI: basic concept.



Caption: Arterial blood is labelled or tagged and, after a delay, moves into the imaging plane or volume, during which time there is T1 decay of the label. Snapshot images are acquired in labelled and control conditions and subtracted, yielding a difference image with intensity proportional to cerebral blood flow (CBF).
Reprinted by permission from Springer Nature © 2007: Neurotherapeutics (Wolf and Detre, 2007).

One issue with ASL is the relatively low signal-to-noise ratio, which means that the difference images need to be collected many times and later averaged in post-processing. This led to development of "pseudo-continuous ASL" (pCASL) which offers enhanced signal-to-noise ratio, superior labelling efficiency and is recommended for clinical imaging by consensus guidelines (Alsop *et al*, 2015). ASL measures are also sensitive to other factors that can affect blood blow, such as use of caffeine, nicotine and drugs of abuse, and are highly sensitive to motion artefacts (i.e. from head movement) and susceptibility artefacts (e.g. from signal dropout at air-tissue interfaces, such as sinuses) (Grade *et al*, 2015). However, a major benefit of ASL is the high degree of correlation between metabolism (e.g. as measured with fluorodeoxyglucose FDG-PET) and perfusion. Thus, if researchers are interested in pathophysiology related to altered energy metabolism (as in proposed models of psychosis onset, where increased glutamate is thought to drive excitation, which increases metabolic demand and thus

blood flow), then such metabolic dysfunction will be reflected by—and can therefore be measured noninvasively by—altered cerebral perfusion.

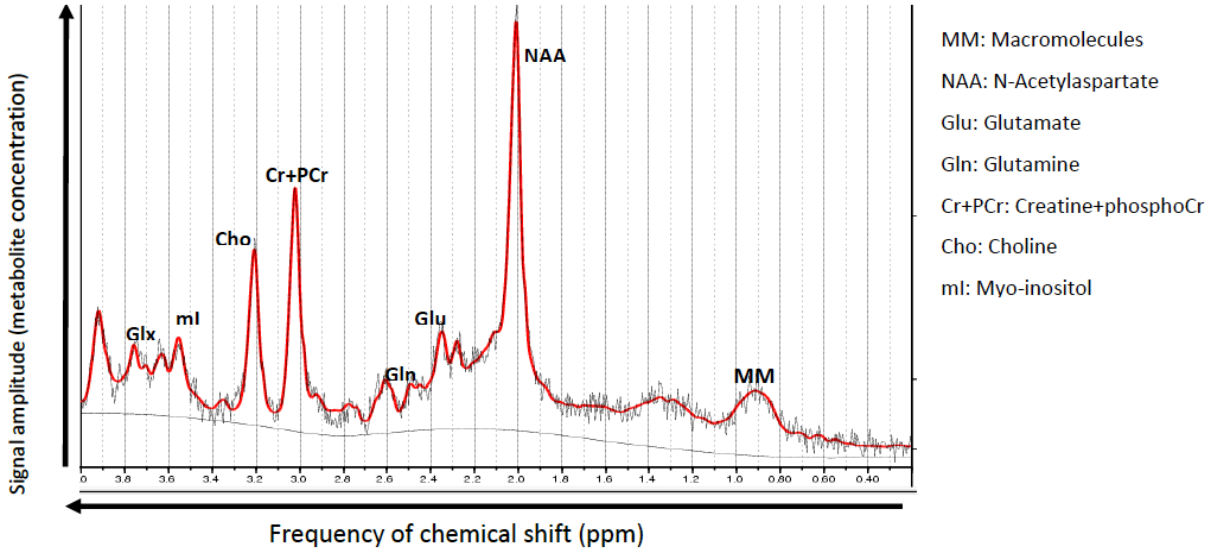
4.4.4. PROTON MAGNETIC RESONANCE SPECTROSCOPY – METHODS

Single voxel 1H-MRS is an MRI technique used to noninvasively quantify the concentrations of tissue metabolites/neurotransmitters within a predetermined voxel of interest. Metabolites must have a concentration of at least 0.5 nanomolar (nM) and typically 0.5-10 millimolar (mM) to be detected (Van Der Graaf, 2010). For this reason, although many chemicals can technically be detected using 1H-MRS, in practice, at 3 Tesla (3T) we are usually restricted to (and interested in) glutamate, glutamate plus glutamine (commonly termed Glx), choline, creatine, n-acetylaspartate (NAA) and myo-inositol. GABA can only be assessed using specific spectral editing techniques (e.g. MEGA-PRESS).

1H-MRS capitalises on the resonant frequency of protons (1H). The main magnet is used to induce a strong static magnetic field (B0), causing protons in the tissue to align themselves in the direction of the applied field (B0) (Van Der Graaf, 2010). In this orientation, the protons are said to be in a low-energy (alpha) state with their magnetic moments aligned parallel to the static magnetic field. The protons precess (spin) about their axis (resonate) at a frequency determined by the Larmor equation, which itself depends on the external magnetic field (B0) and the local chemical environment (i.e. which molecular group is carrying the proton). The molecular group to which a proton belongs affects the local magnetic field because of circulating electron clouds—electrons generate small magnetic fields and thus produce small shifts in precession (resonant) frequency. This is termed chemical shift: the difference in precession frequency resulting from differing chemical environments, which is expressed in units of parts per million (ppm). When a radiofrequency pulse is applied, the protons (still in the alpha state) are excited (absorb energy) and flip into a high-energy (beta) state, with their magnetic moments aligned anti-parallel to the static magnetic field. However, protons can only absorb energy (frequencies) which ‘match’ the difference in energy required to flip between its low and high-energy states (i.e. energy with the resonant frequency). As the protons return to their low-energy state, they emit energy at the resonant frequency which produces a specific signal that can be measured by a receiver coil.

In short, protons in different chemical environments have to absorb, and therefore emit, different resonant frequencies (amounts of energy) to switch between the low and high-energy states. This allows their discrimination and can be plotted in resulting spectra (**Figure 4–4**), with the specific frequency of the chemical shift (ppm) on the x-axis, and with the metabolite concentration (number of absorptions) as signal amplitude on the y-axis.

Figure 4–4. Example Proton Magnetic Resonance Spectroscopy (1H-MRS) spectra.



Volume selection

Volume selection uses 3 slice-selector radiofrequency pulses (in the 3 orthogonal planes, thus exciting a specific 3D volume of tissue) to control the spatial origin of the detected signal. Specifically, a radiofrequency pulse and a gradient pulse are applied in the first direction (e.g. X, which excites a whole slab), then the second direction (e.g. Y, which excites a column from the slab), and finally in the third direction (e.g. Z, which excites a specific cube from the column of the slab)—the intersection of these planes determines the 3D voxel.

Water suppression

Water is the most abundant source of protons in the brain. As such, it produces the greatest spectral peak (amplitude) which obscures the signal from the metabolites of interest. Water suppression methods, such as CHESS (the most common method) apply radiofrequency pulses targeted to the resonant frequency of the water peak, thus eliminating the water signal (Haase *et al*, 1985).

Acquisition sequences

There are a number of different 1H-MRS acquisition sequences for obtaining spectra, with STEAM and PRESS the most common. In short, the difference between them is that STEAM uses 3 pulses all at 90 degrees, while PRESS uses 90, -180 and -180 degree pulses, both again with gradients in each plane for 3D voxel selection (Van Der Graaf, 2010). PRESS has benefits over STEAM in that the signal-to-noise ratio is larger (because the 180-degree pulses give a signal two times the magnitude than that of STEAM), albeit slice selection is worse with PRESS due to the longer echo time required for the 180-degree pulses (Van Der Graaf, 2010). A number of quantification software packages are now available. LCModel is among the most popular; a model is fitted to the data to determine the concentrations of each metabolite (Provencher, 1993). Concentrations are often arbitrary and so scaling to an internal reference (such as creatine, which tends to be stable) is often used, with metabolite levels reported as a ratio to creatine (often denoted "*metabolite*/Cre"). Another approach is to correct the metabolite levels for the proportion of white matter (WM), grey matter (GM) and CSF within the voxel (often called CSF or voxel tissue-corrected) (see **Paper 4** for method).

4.5. SUMMARY, AIMS & HYPOTHESES

Hippocampal hyperperfusion and altered levels of neurochemical metabolites represent two key pathophysiological targets for CHR-P treatment strategies. Previous evidence indicates that oxytocin may have neurophysiological effects within these two domains (Aoki *et al*, 2015; Benner *et al*, 2018; Martins *et al*, 2019; Paloyelis *et al*, 2016). Oxytocin has also been shown to improve symptoms and normalise various indices of brain activation and connectivity in patient populations (as reviewed in section 4.3). However, oxytocin has never been tested in CHR-P individuals, either 'offline' or using MRI. Conducting these experiments in CHR-P groups rather than patients with established psychotic disorders is advantageous because they are (generally) antipsychotic naïve (Fusar-Poli *et al*, 2015b), lack illness chronicity and intervening early (before more severe or enduring neural changes take place) is thought to be the approach with maximum therapeutic potential (Millan *et al*, 2016).

Part 2 of this thesis aims to:

- investigate whether oxytocin can modulate hippocampal perfusion in people at CHR-P using ASL in a double-blind, placebo-controlled, crossover study of acute 40IU intranasal oxytocin (**Paper 3**, starting on page 93).

- evaluate whether oxytocin can modulate concentrations of neurochemical metabolites—particularly glutamate and Glx—in the hippocampus, anterior cingulate cortex and thalamus, using 1H-MRS in double-blind, placebo-controlled, crossover study of 40IU intranasal oxytocin in CHR-P individuals (**Paper 4**, starting on page 118).

Hypotheses:

- ASL study (**Paper 3**)

  Our primary hypothesis was that oxytocin would modulate hippocampal perfusion in CHR-P subjects. We did not hypothesise a specific effect direction because the aim of this study was to demonstrate disease-target (hippocampal) engagement. Secondary predictions were that its effects would be particularly evident in the Cornu Ammonis 1 (CA1) subregion of the hippocampus, and that it would also influence blood flow outside the hippocampus in regions implicated in social and emotional processing.

- 1H-MRS study (**Paper 4**)

  Our primary hypothesis was that oxytocin would modulate glutamate and Glx concentrations (scaled to creatine) in the left hippocampus, anterior cingulate cortex and left thalamus, and our secondary hypotheses tested for effects on other metabolites (myo-inositol, N-acetylaspartate and choline) in the same regions.

5. OXYTOCIN – ASL STUDY

5.1. PAPER 3 – OXYTOCIN ASL

Davies C, Paloyelis Y, Rutigliano G, Cappucciati M, De Micheli A, Ramella-Cravaro V, et al (2019). Oxytocin modulates hippocampal perfusion in people at clinical high risk for psychosis. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-018-0311-6

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ARTICLE OPEN

Oxytocin modulates hippocampal perfusion in people at clinical high risk for psychosis

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Preclinical and human studies suggest that hippocampal dysfunction is a key factor in the onset of psychosis. People at Clinical High Risk for psychosis (CHR-P) present with a clinical syndrome that can include social withdrawal and have a 20–35% risk of developing psychosis in the next 2 years. Recent research shows that resting hippocampal blood flow is altered in CHR-P individuals and predicts adverse clinical outcomes, such as non-remission/transition to frank psychosis. Previous work in healthy males indicates that a single dose of intranasal oxytocin has positive effects on social function and marked effects on resting hippocampal blood flow. The present study examined the effects of intranasal oxytocin on hippocampal blood flow in CHR-P individuals. In a double-blind, placebo-controlled, crossover design, 30 CHR-P males were studied using pseudo-continuous Arterial Spin Labelling on 2 occasions, once after 40IU intranasal oxytocin and once after placebo. The effects of oxytocin on left hippocampal blood flow were examined in a region-of-interest analysis of data acquired at 22–28 and at 30–36 minutes post-intranasal administration. Relative to placebo, administration of oxytocin was associated with increased hippocampal blood flow at both time points ($p = .0056$; $p = .034$), although the effect at the second did not survive adjustment for the effect of global blood flow. These data indicate that oxytocin can modulate hippocampal function in CHR-P individuals and therefore merits further investigation as a candidate novel treatment for this group.

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INTRODUCTION

At present, there is a lack of effective treatments for individuals at Clinical High Risk of Psychosis (CHR-P [1]). Recent studies suggest that existing interventions do not significantly impact on transition to psychosis [2], attenuated positive [3] or negative symptoms [4], or social and functional outcomes [5]. Novel treatments for this population are therefore needed [6].

A substantial body of scientific work places aberrant hippocampal structure and function at the core of neurobiological mechanisms underlying the onset of psychosis [7]. Evidence from post-mortem, neuroimaging and preclinical research suggests that the onset of attenuated psychotic symptoms may be driven by dysregulated glutamate neurotransmission in the Cornu Ammonis 1 (CA1) region of the hippocampus, which is thought to lead to hypermetabolism and altered (increased) blood flow [7–10]. Enhanced glutamatergic tone in CA1 induces allostatic adaptations [11] in γ-aminobutyric acid (GABA)-ergic neurotransmission, with consequent disinhibition of pyramidal neurons (Fig. 1) [12]. These changes may lead to disturbed neural excitation/inhibition balance, and via polysynaptic projection pathways to the midbrain/striatum, to midbrain hyperdopaminergia [13, 14]. As the CHR-P state progresses to the first episode of psychosis, the functional perturbations once localised to (particularly the left) CA1 may spread to extra-hippocampal regions such as the frontal cortex [7, 8], and excitotoxic as well as atrophic processes culminate in hippocampal volume loss—structural changes—beginning in CA1 [15–17]. These findings are consistent with evidence that CHR-P individuals show increased resting regional cerebral blood flow (rCBF) in the hippocampus relative to controls [18, 19], and normalisation (reduction) of left hippocampal rCBF is associated with remission from the CHR-P state [18]. Hippocampal rCBF in CHR-P individuals has also been correlated with cortical GABA levels [20].

The neuropeptide oxytocin is a key modulator of social, sexual and emotional processes [21], including hypothalamic-pituitary-adrenal axis regulation [22], emotion recognition [23], social memory [24] and reward, and possesses anxiolytic [21, 22] and prosocial properties [25, 26]. Previous work in healthy males [27]

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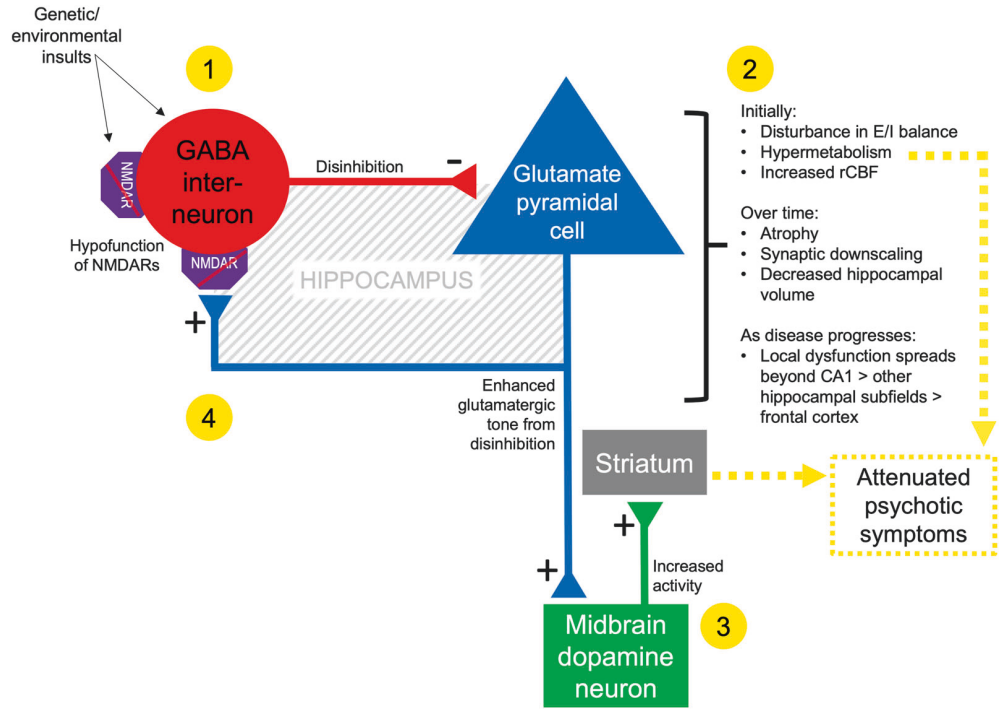

Fig. 1 Simplified schematic of proposed neural circuit mechanisms of hippocampal pathophysiology in those at CHR-P. In (**1**), low glutamate signal/input from hypofunctioning NMDARs (akin to 'faulty homeostatic sensors') leads GABAergic interneurons to seek to homeostatically increase excitation by reducing inhibition (disinhibition) of glutamatergic pyramidal cells. However, by disinhibiting pyramidal cells (and thus increasing glutamate signalling) in this dysfunctional neural environment, the potential homeostatic adaptation becomes allostatic (**2**). In (**3**), enhanced excitation leads to an overdrive in the responsivity of midbrain dopamine neurons which project to the associative striatum (note that the connection between hippocampal pyramidal cells and midbrain dopamine neurons is presented as monosynaptic but is actually polysynaptic via the ventral striatum and ventral pallidum). Completing the (simplified) circuit, local glutamatergic tone is increased in (**4**) but is not detected as such by hypofunctioning NMDARs on GABAergic interneurons. For detailed original diagrams and discussion of evidence for this proposed circuit or its component processes, see [7, 11, 12, 14, 74]. *Glu* glutamate, *NMDAR* N-methyl-D-aspartate receptor, *E/I* excitation/inhibition

suggests that an acute dose of intranasal oxytocin significantly increases rCBF in limbic and midbrain regions, including the hippocampus. Preclinical studies have also identified the hippocampus as a key target for oxytocin-mediated effects, with oxytocin enhancing the signal-to-noise ratio of CA1 pyramidal cell firing by selectively targeting GABAergic interneuron function [28, 29]—part of the neural circuit implicated in psychosis onset [6, 12, 14]. Oxytocin is further linked to these mechanisms via its role in neural circuit maturation in the pre- and peri-natal period, responsible for the excitatory-to-inhibitory switching of GABAergic signalling [30] and controlling dendrite complexity [31], synapse density [31] and the onset of synchronous firing in developing hippocampal pyramidal neurons [32]. Preclinical research also indicates that oxytocin can protect hippocampal CA1 plasticity and memory from the effects of stress [33], which is also implicated in the onset of psychosis [13].

The aim of the present study was to examine the acute effects of oxytocin on hippocampal rCBF in CHR-P individuals. We focused on the left (and not right) hippocampus because previous CHR-P research has repeatedly implicated the left hippocampus in the pathophysiology of psychosis risk [7, 8, 10, 18, 20, 34, 35], with left (and not right) hippocampal rCBF associated with clinical outcomes [18]. In addition, previous oxytocin research has reported distinctly left-lateralised effects of oxytocin on cerebral blood flow [27]. In view of evidence that certain subregions of the hippocampus may be particularly involved in the risk for psychosis, and may be particularly influenced by oxytocin, we also investigated whether the effects of oxytocin were specific to different hippocampal subregions. Finally, we explored whether oxytocin had additional effects outside the hippocampal region in a whole-brain analysis. The a priori evidence for altered hippocampal rCBF in CHR-P individuals vs controls comes from the same clinical sample as the current study—recruited from the Outreach And Support in South London (OASIS) service [36]—and has already been replicated in a further sample recruited from this clinic [18, 19]. Leveraging these findings, the current study adopted a within-subject crossover design. Our first hypothesis was that oxytocin would modulate hippocampal rCBF in CHR-P subjects. We did not hypothesise a specific effect direction because the aim of this study was to demonstrate disease-target (hippocampal) engagement. Secondary predictions were that its effects would be particularly evident in the CA1 subregion, and that it would also influence rCBF outside the hippocampus in regions implicated in social and emotional processing.

PATIENTS & METHODS

Participants

The study received National Research Ethics Service approval (14/LO/1692) and all subjects gave written informed consent. Thirty male, help-seeking CHR-P individuals aged 18–35 were recruited from two specialist early detection services—the OASIS [36] and Tower Hamlets Early Detection Service (THEDS). A CHR-P status was determined using the Comprehensive Assessment of At-Risk Mental States (CAARMS) 12/2006 criteria [37]. Briefly, subjects met one or more of the following subgroup criteria: (a) attenuated psychotic symptoms, (b) brief limited intermittent psychotic symptoms (BLIPS, psychotic episode lasting < 1 week, remitting

without treatment), or (c) either schizotypal personality disorder or first-degree relative with psychosis [37], all coupled with functional decline. Individuals were excluded if there was a history of previous psychotic disorder (with the exception of BLIPS, some of whom may meet acute and transient psychotic disorder criteria [38]) or manic episode, exposure to antipsychotics, neurological disorder or current substance-use disorder, estimated IQ < 70, acute intoxication on the day of scanning, and any contraindications to magnetic resonance imaging (MRI) or intranasal oxytocin or placebo. History of Axis I disorder(s) was not an exclusion criterion due to the transdiagnostic nature of the CHR-P state and the high prevalence of such diagnoses within these populations [39].

Design, materials, procedure

We used a randomised, double-blind, 40 IU intranasal oxytocin vs placebo single-dose challenge in a crossover design (1-week wash out). During each challenge, subjects underwent an MRI scan which started at 1130 h to minimise potential effects of diurnal variation in oxytocin or vasopressin [27]. Anxiety was measured using the State-Trait Anxiety Inventory (STAI) prior to each scan (and prior to intranasal administration) so that pre-scan anxiety score could be included in statistical models as a covariate. For descriptive purposes, we also collected information on medication history, use of alcohol (Alcohol Use Disorders Identification Test), tobacco and cannabis, functioning using the Global Functioning Role and Social scales [40] and later transition status. Intranasal administration followed recommended guidelines and a protocol adopted by a previous study conducted at our institute [27]. Briefly, participants self-administered one puff (4 IU) of intranasal oxytocin or matched placebo every 30 s, alternating between nostrils, until 40 IU had been administered (Supplementary Materials and Methods). During the scan, participants were asked to maintain their gaze on a centrally-placed fixation cross.

MRI acquisition and image processing

All scans were conducted on a General Electric Discovery MR750 3 Tesla system (General Electric, Chicago, USA) using a 32-channel head coil. Measurement of Cerebral Blood Flow (CBF) was carried out using a 3D pseudo-continuous Arterial Spin Labelling (3D-pCASL) sequence during two consecutive runs: 22–28 (run 1) and 30–36 (run 2) min post-intranasal administration. The timing of the two runs was selected based on previous findings of the spatiotemporal profile of oxytocin-induced cerebral blood flow changes in healthy males, which demonstrated sustained effects over a ~20–73 min period (post-intranasal administration) [27]. For each subject, we also computed a mean (average) CBF map from the CBF maps for runs 1 and 2. ASL data were preprocessed using the Automatic Software for ASL Processing (ASAP) 2.0 toolbox [41] running in Statistical Parametric Mapping version 12 (SPM12; https://www.fil.ion.ucl.ac.uk/spm/) on Matlab R2017a. 3D-pCASL acquisition parameters and image preprocessing procedures were conducted in line with previous studies and are detailed in the Supplementary Materials and Methods.

Statistical analysis

Statistical analyses were performed in STATA SE14.2.

Pre-scan anxiety scores

For pre-scan anxiety (STAI) scores, missing data were imputed using next-observation-carried-backward (Supplementary Materials and Methods). Differences in pre-scan anxiety scores in the oxytocin vs placebo conditions was assessed using a paired t-test. In line with previous CHR-P studies [18, 19] and because anxiety has been demonstrated to have systematic effects on CBF [42] (including rCBF specifically in the hippocampus [43]), all analyses included mean-centred pre-scan anxiety as a covariate.

Global cerebral blood flow (CBF)

To measure global CBF signal, we used the ASAP toolbox to extract average CBF values from a grey matter mask for each subject. The ICBM-152 mask was obtained from the DARTEL toolbox in SPM and thresholded to contain voxels with a >.25 probability of being grey matter. To ascertain whether global CBF was significantly different in the oxytocin relative to placebo condition, we conducted repeated-measures analyses of covariance (RM-ANCOVA) in STATA for run 1, run 2, and the mean of the runs (separately), using pre-scan anxiety as covariate (Supplementary Materials and Methods). All subsequent analyses were conducted with and without global CBF as covariate.

Hippocampal ROI rCBF

Effects of oxytocin on hippocampal rCBF were determined using a region-of-interest (ROI) approach. A left hippocampal ROI was defined anatomically in MNI space using the cytoarchitectonic probabilistic atlas [44] as implemented in the Anatomy toolbox [45] in SPM (Figs. 2a, b). The ROI mask was composed of regions CA1, CA2, CA3, dentate gyrus, and subiculum. Mean rCBF values for the ROI were extracted for each subject using ASAP toolbox and entered into RM-ANCOVAs in STATA (Supplementary Materials and Methods). Our primary analyses tested for effects in each of the two runs separately. However, due to the low signal-to-noise ratio inherent in ASL data, we also conducted an analysis of the mean effect across the two runs, which can help to reduce noise if the effects are stable [27]. We contained the family-wise error (FWE) rate at $\alpha = .05$ using the Hochberg procedure, which is a 'sharper' and more powerful version of the Bonferroni adjustment and which allows non-independence between statistical tests [46]. Original p values (two-tailed) are reported alongside indication of Hochberg correction survival (i.e., whether or not they remain significant (survive) after accounting for FWE). Effect sizes are reported as omega-squared (ω^2).

Exploratory/supplemental analyses

We used analogous procedures to those described directly above to extract mean rCBF values for each hippocampal subregion, using separate masks for left CA1, CA2, CA3, dentate gyrus, and subiculum (Fig. 3a; and Supplementary Materials and Methods). No multiplicity correction was applied as subregion analyses were exploratory. Finally, for completeness we examined whole-brain effects in runs 1, 2 and the mean of the runs—separately—using paired t-tests (second-level analysis) in SPM, with pre-scan anxiety and with/without global CBF signal as nuisance covariates. We conducted a whole-brain search using cluster level inference (cluster forming threshold: $p < .005$; cluster reported as significant at $p < .05$ using FWE correction in SPM). Analyses were restricted using the explicit ICBM mask again thresholded to contain voxels with >.25 probability of being grey matter.

RESULTS

Sample characteristics

Demographic and clinical characteristics of the sample are presented in Table 1. All participants completed the study with no drop-outs. No adverse side effects were clinically observed. One subject was removed due to protocol violations, leaving a sample of $N = 29$. There was a significant difference in pre-scan (pre-intranasal administration) anxiety scores in the oxytocin vs placebo condition (oxytocin [mean ± SE] = 37.4 ± 1.9; placebo = 33.4 ± 1.7; $t(28) = 2.46$, $p = .020$), which may have arisen by chance or due to slightly more individuals receiving treatment order oxytocin > placebo ($N = 15$) vs placebo > oxytocin ($N = 14$).

Global CBF

There was no significant difference in global grey matter CBF values (ml/100 g/min) in the oxytocin relative to the placebo condition in

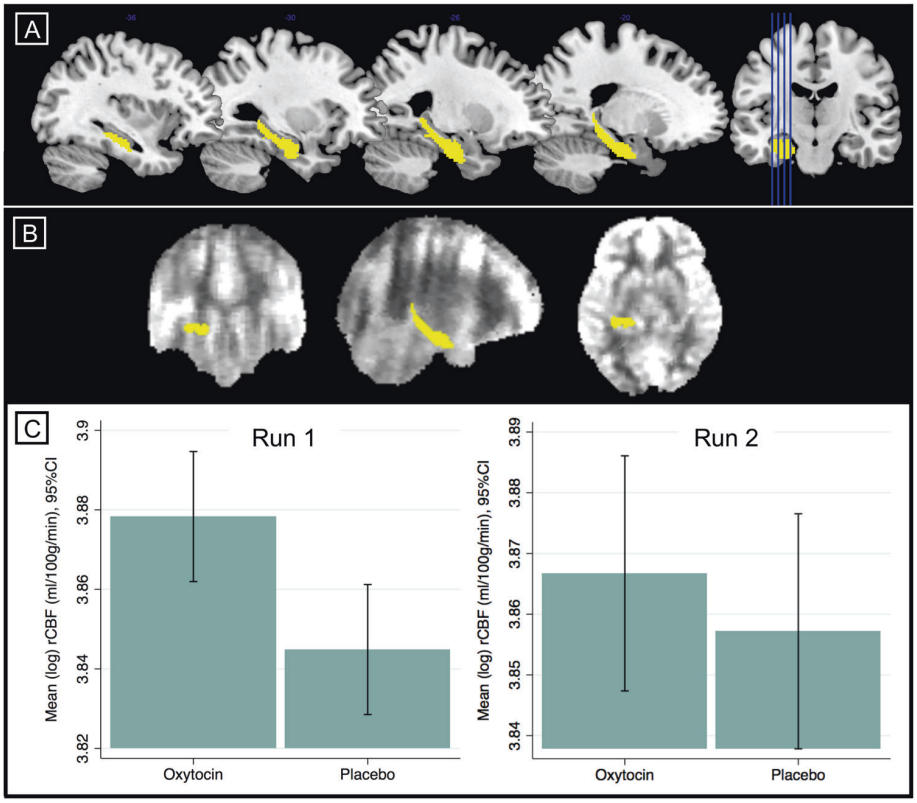

Fig. 2 rCBF Effects in Left Hippocampus. **a** ROI mask for the left hippocampus (yellow) overlaid on a standard brain template, and (**b**) overlaid on a representative subject-level cerebral blood flow map in normalised space, and (**c**) bar charts showing mean hippocampal rCBF in the oxytocin and placebo conditions in run 1 and run 2 after adjustment for global effects

the mean of both runs (oxytocin [marginal mean ± SE] = 52.91 ± 0.91; placebo = 50.23 ± 0.91; $F(1,27) = 4.00$, $p = .056$) or run 1 (oxytocin = 52.89 ± 0.94; placebo = 50.44 ± 0.94; $F(1,27) = 3.14$, $p = .088$), but a significant difference was observed in run 2 (oxytocin = 52.94 ± 0.90; placebo = 50.03 ± 0.90; $F(1,27) = 4.75$, $p = .038$).

Hippocampal rCBF

rCBF values for all runs were log transformed due to deviations from distributional assumptions for parametric tests. Compared to placebo, oxytocin administration was associated with increased hippocampal rCBF in run 1 ($F(1,27) = 9.06$, $p = .0056$; $\omega^2 = .223$), run 2 ($F(1,27) = 4.96$, $p = .034$; $\omega^2 = .124$) and the mean of the two runs ($F(1,27) = 7.31$, $p = .012$; $\omega^2 = .184$), all of which survived Hochberg multiplicity correction (Figure S1). After controlling for global signal effects, oxytocin administration was associated with increased hippocampal rCBF in run 1 ($F(1,26) = 7.68$, $p = .010$; $\omega^2 = .198$) which survived multiplicity correction (Fig. 2c). The effects were no longer evident in run 2 ($F(1,26) = 0.44$, $p = .51$; Fig. 2c) or in the mean of the runs ($F(1,26) = 3.27$, $p = .082$). Exclusion of participants taking antidepressants ($N = 8$) and benzodiazepines ($N = 1$) in sensitivity analyses made no material change to the unadjusted effects on hippocampal rCBF (Supplementary Materials and Methods).

Exploratory/supplemental analyses

Hippocampal subregions. Hippocampal subregion effects were explored in run 1 only. Relative to placebo, oxytocin administration was associated with increased rCBF in all hippocampal subregions, including CA1 ($F(1,27) = 9.44$, $p = .0048$; $\omega^2 = .232$), CA2 ($F(1,27) = 9.33$, $p = .0050$; $\omega^2 = .229$), CA3 ($F(1,27) = 6.83$, $p = .014$; $\omega^2 = .172$), subiculum ($F(1,27) = 7.61$, $p = .010$; $\omega^2 = .191$) and particularly the dentate gyrus ($F(1,27) = 10.11$, $p = .0037$; $\omega^2 = .246$)(Figure S2). After controlling for global CBF effects, oxytocin administration was associated with increased rCBF in CA1 ($F(1,26) = 7.29$, $p = .012$; $\omega^2 = .189$), CA2 ($F(1,26) = 6.32$, $p = .018$; $\omega^2 = .165$), subiculum ($F(1,26) = 6.03$, $p = .021$; $\omega^2 = .157$) and dentate gyrus ($F(1,26) = 7.40$, $p = .011$; $\omega^2 = .192$), but no difference was found in CA3 ($F(1,26) = 3.20$, $p = .086$)(Fig. 3b). As noted above, these results were not corrected for multiple comparisons.

Whole-brain. Since there was no significant difference in global signal, unadjusted whole-brain results (including for the mean of the runs) are reported in Table 2 (see Table S1 and Supplementary Material for global signal-adjusted results). In run 1, oxytocin administration was associated with increased perfusion in a large predominantly left-lateralised cluster spanning the cerebellum, hippocampus, parahippocampal gyrus and visual cortex, with a peak in the cerebellum ($p_{FWE} < .05$). There were no regions where perfusion decreased after oxytocin. In run 2, oxytocin was associated with increased perfusion in a large left-hemisphere cluster spanning the thalamus, parahippocampal gyrus, hippocampus, and fusiform gyrus, with a peak in the parahippocampal gyrus ($p_{FWE} < .05$), and in a separate right-hemisphere cluster with a peak in the superior parietal lobule ($p_{FWE} < .05$).

DISCUSSION

This is the first study to investigate the neurophysiological effects of oxytocin in CHR-P individuals. The key finding was that a single dose of intranasal oxytocin increased resting cerebral perfusion in the hippocampus, a region critically implicated in the pathophysiology of the CHR-P state and the later onset of psychosis. This

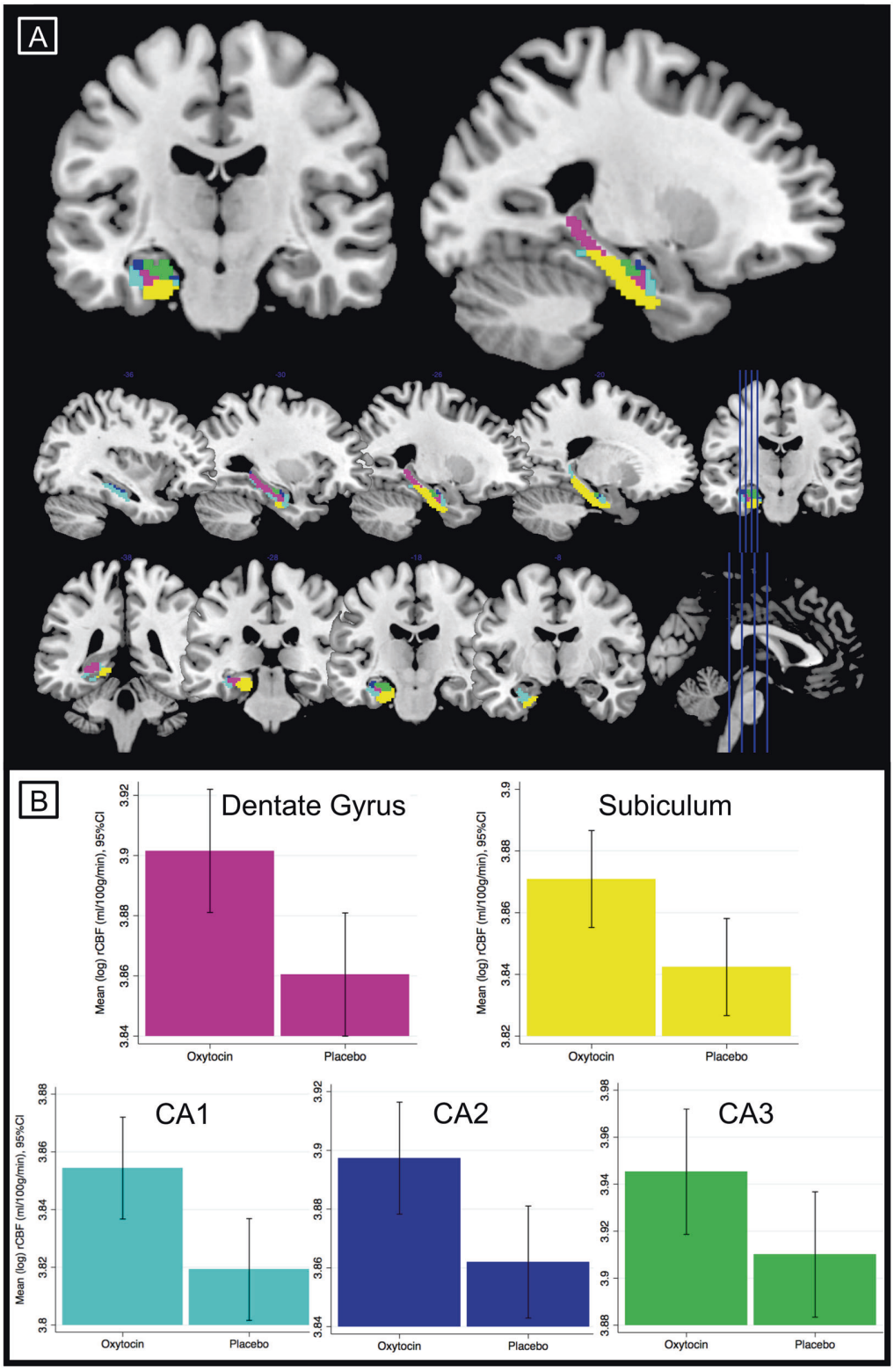

Fig. 3 rCBF in Left Hippocampal Subregions. **a** ROI masks for left hippocampal subregions: dentate gyrus (pink), subiculum (yellow), CA1 (cyan), CA2 (blue), and CA3 (green) displayed on a standard brain template, and (**b**) bar charts showing mean hippocampal subregion rCBF in the oxytocin and placebo conditions in run 1 after adjustment for global effects

Table 1. Participant demographic and clinical characteristics

	Variable	Total sample ($N = 30$)
Demographic	Age, years; mean (SD)	23.2 (4.7)
	Age range, years	18–35
	Sex, male/female	30/0
	Ethnicity (White/Black/Asian/Mixed)	16/6/4/4
	Handedness, right/left	26/4
	Education, years; mean (SD)	13.2 (1.9)
Clinical	CHR-P Subtype[a] (BLIPS/APS/GRD)	6/23/1
	CAARMS attenuated positive symptoms[b]; mean (SD)	11.7 (3.3)
	Transition to psychosis (yes/no)[c]	4/26
	Baseline anxiety score[d]; mean (SD)	35.6 (8.7)
	GF social score; mean (SD)	6.8 (1.5)
	GF role score; mean (SD)	7.0 (1.7)
	Current antidepressant medication (yes/no)	8/22
	Current antipsychotic medication (yes/no)	0/30
	Current benzodiazepine medication (yes/no)	1/29
Substance Use	Current smoker (yes/no)	17/13
	Cigarettes/day; mean (SD)	9.8 (6.0)
	Cannabis use[e]; median (range)	2 (0–4)
	Alcohol, AUDIT total; mean (SD)	7.2 (7.7)

[a]Comprehensive Assessment of At-Risk Mental States (CAARMS) subgroup; *BLIPS* brief limited intermittent psychotic symptoms, *APS* attenuated psychotic symptoms, *GRD* genetic risk and deterioration
[b]Sum of the global (severity) ratings for positive subscale items (P1-P4) of the CAARMS
[c]The 4 transitions occurred within 26 months but the follow up is still ongoing
[d]Mean of pre-scan anxiety scores across conditions as measured by the State Trait Anxiety Inventory (STAI)
[e]Cannabis use: 0 = never, 1 = experimental use (tried occasionally), 2 = occasional use (small quantities from time to time), 3 = moderate use (moderate quantities regularly / large amounts occasionally), 4 = severe use (frequently used large quantities, often to intoxication/debilitation). *AUDIT* alcohol use disorders identification test, *CHR-P* clinical high risk for psychosis, *GF* global functioning (role and social) scale

finding is consistent with the only previous study of the effects of oxytocin on rCBF, which found left-lateralised increases in a large limbic cluster which included the hippocampus [27]. Our analysis of hippocampal subregions indicated that the largest effects of oxytocin were in the dentate gyrus and CA1 (although these analyses were exploratory and require confirmation and replication), while whole-brain analysis showed that oxytocin also modulated perfusion in the thalamus, parietal cortex and cerebellum.

Altered (increased) cerebral blood flow represents a core pathophysiological mechanism for psychosis onset [7–9] and is one of the few neuroimaging findings to have been replicated in independent CHR-P samples [8, 18, 19]. Increased hippocampal activity is also a key feature of preclinical models of psychosis [8, 12] and is thought to drive subcortical dopamine dysfunction [14]. Increases in hippocampal rCBF may therefore represent a disease-modifying target [7]. In view of this literature, our analyses focused on the hippocampal region. The left side (alone) was selected because previous CHR-P research has repeatedly implicated the left hippocampus in the pathophysiology of psychosis risk [7, 8, 10, 18, 20, 34, 35], with left (and not right) hippocampal blood flow associated with clinical outcomes (i.e., remission from a CHR-P state vs non-remission/transition to psychosis) [18]. In addition, previous oxytocin research has reported distinctly left-lateralised effects of oxytocin on cerebral blood flow [27].

We found that the effect of oxytocin on hippocampal rCBF in run 2 became non-significant after controlling for global signal. This may have reflected poorer signal-to-noise ratio in run 2 than run 1, as inspection of the raw data suggested there was greater variance in both hippocampal rCBF and global CBF values. Another possibility is that it was related to the much more pronounced effects of oxytocin on global rCBF during run 2 than in run 1. A final consideration is that our findings were influenced by the time course and dose-response effects of oxytocin, which may follow an inverted U-shaped curve [47, 48].

In exploratory analyses, we found that oxytocin increased rCBF in all of the hippocampal subregions that were examined, with the largest effects in the dentate gyrus and in CA1 (other subregions are discussed in the Supplementary Material). Previous neuroimaging research in CHR-P individuals suggests that CA1 is a key locus of dysfunction [8, 15]. The CA1 region plays an integral role in social and autobiographical memory [49] and CHR-P individuals show impairments in these domains [50]. In healthy individuals, oxytocin enhances social learning [51] and memory [24]. CA1 dysfunction is also at the centre of pathophysiological processes implicated in the onset of psychosis [7]; transition and/or non-remission from a CHR-P state is associated with enhanced CA1 perfusion and hypermetabolism [8, 18] and a gradual decline in CA1 volume [15]. Compared to other hippocampal subregions, CA1 has the highest number of GABAergic interneurons [15, 52] and an N-methyl-D-aspartate receptor (NMDAR) expression profile which confers enhanced susceptibility to glutamatergic alterations and excitotoxicity [53]—key features of the proposed neural circuit underlying psychosis onset [7]. In preclinical studies, oxytocin modulates this neural circuit by targeting GABAergic interneuron function and enhancing the signal-to-noise ratio of CA1 pyramidal cell firing [28, 29].

In terms of the dentate gyrus, CHR-P individuals whose symptoms had remitted were recently shown to have a longitudinal reduction in left hippocampal perfusion [18], which reference to a cytoarchitectonic atlas [44, 45] indicates had its peak coordinate in the left dentate gyrus. Another study reported reduced dentate gyrus volumes in CHR-P patients vs controls [17]. The dentate gyrus is thought to function as a computational pattern separator, with dysfunction here mechanistically linked to NMDAR hypofunction [54] and generation of spurious associations that may contribute to the onset of psychotic symptoms [55]. Patients with first-episode psychosis show deficits in pattern separation, which can be recreated in healthy volunteers using ketamine (NMDAR antagonist) challenge [54]. Interestingly, oxytocin is thought to exert its facilitatory effects on social recognition and behaviour via oxytocin receptors in the dentate gyrus, which recruit pattern separation circuits to minimise interference between similar social memories—at least in preclinical models [56]. In rats, activation of oxytocin receptors drives GABA release in the dentate gyrus in an action potential-dependent manner [57], and exogenous oxytocin has stimulatory effects on cell proliferation and adult neurogenesis, even under conditions of stress and elevated glucocorticoids, which also appears to be specific to the dentate gyrus [58]. These findings further demonstrate that oxytocin engages key pathophysiological circuits that are associated with the onset of psychosis.

We also investigated the effects of oxytocin at the whole-brain level. We found that oxytocin was associated with increased perfusion in large clusters spanning the hippocampus, parahippocampal gyrus and fusiform gyrus, as well as the cerebellum, and in run 2, the thalamus. Effects in these regions are consistent with previous work on (a) the effects of oxytocin on perfusion in

Table 2. Effects of oxytocin vs placebo on whole-brain CBF (without adjustment for global CBF effects)

Cluster Description	Hemisphere	*k*	$P_{(FWE\text{-}corr)}$	Peak coordinates			Peak description
				x	y	z	
Run 1, Oxytocin > Placebo							
Left cerebellum, visual cortex, parahippocampal gyrus, hippocampus, fusiform gyrus, lingual gyrus; right cuneus, calcarine gyrus, visual cortex, cerebellum	Left	3904	<.05	−26	−32	−36	Cerebellum (culmen)
				−20	−46	−28	Cerebellum (culmen)
				−24	−72	−2	Lingual gyrus
Run 1, Placebo > Oxytocin							
None							
Run 2, Oxytocin > Placebo							
Left cerebellum, fusiform gyrus, parahippocampal gyrus, hippocampus, lingual gyrus, thalamus; right cerebellum	Left	3117	<.05	−36	−44	−8	Parahippocampal gyrus
				−2	−80	−34	Cerebellum (pyramis)
				−16	−58	−12	Cerebellum (culmen)
Right superior parietal lobule, precuneus, calcarine gyrus, cuneus, visual cortex; left visual cortex	Right	2394	<.05	22	−60	68	Superior parietal lobule
				8	−48	74	Postcentral gyrus
				4	−82	28	Cuneus
Run 2, Placebo > Oxytocin							
None							
Mean of the runs, Oxytocin > Placebo							
Left cerebellum, parahippocampal gyrus, hippocampus, fusiform gyrus, thalamus, lingual gyrus, visual cortex; right cuneus, visual cortex, cerebellum	Left	5348	<.05	−26	−32	−36	Cerebellum (culmen)
				−26	−48	20	White matter
				−30	−48	10	White matter
Mean of the runs, Placebo > Oxytocin							
None							

k number of voxels in the cluster, p_{FWE} FWE-corrected *p*-value

healthy individuals [27], (b) high levels of oxytocin pathway gene expression and mRNA in the hippocampus, parahippocampal gyrus and thalamus (preprint [59]), and (c) the role of these regions in emotion processing and social cognition [21, 60, 61]. Increased perfusion was observed—albeit as part of a large cluster—in the left posterior hippocampus (including CA1 and dentate gyrus) at the whole-brain level across all runs, despite not surviving adjustment for global signal effects. The left-lateralised temporal lobe findings are in line with previous oxytocin work [27, 47, 60, 62] and predominantly left-hemisphere ROIs used in CHR-P neuroimaging studies [34, 63].

A separate healthy control group was not included in the current study because two previous independent CHR-P samples recruited from the same clinical service—the OASIS—have shown that hippocampal perfusion is altered in CHR-P individuals vs controls. These studies were large and the findings replicated, thus providing a priori evidence of hippocampal rCBF alterations in CHR-P individuals. Thus, we have used ROIs based on previous studies that reflect validated CHR-P vs control differences. However, future studies that include a parallel group of healthy volunteers would allow examination of the specificity and potential differential effects of oxytocin in CHR-P vs normative samples, as well as aiding the interpretation of the direction (increase vs decrease) of cerebral blood flow effects. Because we only tested one relatively mid-to-high range dose of oxytocin (40IU, which may be sufficient to cross-react with vasopressin receptors to give a vasopressin-like effect [64, 65]), we were not able to evaluate whether lower doses would show different effects (i.e., reduction of hippocampal perfusion). Given that previous studies have reported increased hippocampal perfusion in people at CHR-P [8, 18, 19], it may well be that a reduction in perfusion is the ultimate therapeutic target. These investigations were not possible in the current study, which was primarily an acute challenge to demonstrate disease-target engagement, but they provide the first evidence that intranasal oxytocin can alter cerebral blood flow in CHR-P individuals in target brain regions. Furthermore, while initial evidence of direct nose-to-brain transport has recently emerged [66], the exact mechanism by which it enters the brain is not fully understood, and differences in nasal anatomy and administration technique could influence the amount of oxytocin that reaches the brain. Our crossover (and counterbalanced order) design helped to control for this, but future research could use novel devices which may provide a more consistent and optimised delivery of oxytocin. Although none of the CHR-P participants were taking antipsychotic medication, a minority were taking antidepressants ($N = 8$) or benzodiazepines ($N = 1$), which could have affected the results. However, excluding these subjects did not alter the main results. We also excluded female subjects due to sexual dimorphism in oxytocinergic function [48, 60]. We did not include specific behavioural or symptom data because the study was designed (and therefore powered) to investigate the neurophysiological basis for the effects of oxytocin and to primarily show disease-target engagement. Finally, findings in CHR-P subjects can be influenced by sampling biases that modulate the level of risk for psychosis [67], but the level of risk in subjects from our local CHR-P clinic [36] has remained stable over recent years [68].

CHR-P individuals show deficits in social cognition [69] and altered neural responses during social and emotion processing fMRI tasks [70], which may contribute to a reduction in social and occupational functioning. Because oxytocin can have prosocial effects in healthy volunteers [23, 25] and in patients with schizophrenia [71], and modulates brain activation during social and emotion processing fMRI paradigms [72], this suggests that it

might—subject to future research—be useful as a novel treatment in CHR-P subjects. However, while our results are promising in showing that oxytocin can engage brain regions strongly implicated in the onset of psychosis, they do not tell us about effects on symptoms, functioning, social cognition or any other CHR-P presentation, which limits the clinical interpretability of our findings. These outcomes remain important avenues for future research and we envisage that this study will provide the neurophysiological evidence in support of future longer-term clinical trials that can provide clinical validation. Furthermore, oxytocin has a good side effect profile; it is safe and well tolerated [73] and none of our participants reported adverse effects. At present, there are no licensed pharmacological treatments for this group, and although psychological interventions have been recommended, there is limited evidence that these are effective [2, 3]. Developing effective treatments for CHR-P subjects thus represents an unmet clinical need.

CONCLUSIONS

The present study indicates that a single dose of oxytocin can significantly modulate hippocampal perfusion in people at CHR for psychosis. This suggests that oxytocin merits further investigation as a candidate novel treatment for this group.

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ADDITIONAL INFORMATION

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5.2. SUPPLEMENTARY MATERIAL

OXYTOCIN MODULATES HIPPOCAMPAL PERFUSION IN PEOPLE AT CLINICAL HIGH RISK FOR PSYCHOSIS

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– SUPPLEMENTARY MATERIAL –

Methods

Design, Materials, Procedure

Subjects were asked to abstain from using recreational drugs for at least one week prior to each MRI scan, and alcohol for at least 24 hours prior to each MRI scan. Urine screening was conducted before the scan for each participant.

The blinded spray bottles used for each session (containing oxytocin or matched placebo) were visually identical and dispensed by the Maudsley Hospital Pharmacy. Intranasal administration followed recommended guidelines [6] and a protocol adopted by a previous study conducted at our institute [7]. After a demonstration of the intranasal administration from a researcher using a spray bottle containing water, participants self-administered (in the presence of and with feedback from a researcher) one puff (4IU) of intranasal oxytocin (Syntocinon) or placebo every 30 seconds, alternating between nostrils, until 40IU (10 puffs) had been administered. The administration phase lasted approximately 4.5 minutes. A timer was started after the last puff had been administered and was used to coordinate the start of the first MRI sequence (see section below) run at 22 minutes post-administration (run 1), followed by the second run at 30 minutes (run 2).

Both the participants and researchers were blind to the (crossover) treatment sequence (AB or BA) allocation. A randomisation list was generated by the Maudsley Hospital Pharmacy, which determined whether a participant received oxytocin or placebo in their first study visit and vice versa for the second study visit. On recruitment of a study participant, an unblinded clinical trial pharmacist, who was not involved with the rest of the study, allocated the participant to one of the two sequences (AB, BA) based on the randomisation list. Allocation information was kept concealed in the Maudsley Hospital Pharmacy.

MRI acquisition parameters and procedures

All scans were conducted on a General Electric Discovery MR750 3 Tesla system (General Electric, Chicago, USA) at the Institute of Psychiatry, Psychology and Neuroscience, King's College London, using a 32-channel head coil. Measurement of Cerebral Blood Flow (CBF) was carried out using a 3D pseudo-continuous Arterial Spin Labeling (3D-pCASL) sequence during two consecutive runs, from 22-28 (run 1) and 30-36 (run 2) minutes post-intranasal administration.

Labelling of arterial blood was achieved with a 1525ms train of Hanning-shaped radio frequency pulses in the presence of a net magnetic field gradient along the flow direction (the z-axis of the magnet). After a post-labelling delay of 2025ms, a whole brain volume was read using a 3D inter-leaved "stack-of-spirals" Fast Spin Echo readout [8], consisting of 8 interleaved spiral arms in the in-plane direction, with 512 points per spiral interleave. TE=11ms, TR=5135ms, and 56 slice-partitions of 3mm thickness were defined in the 3D readout. The in-plane FoV was 240×240mm. The spiral sampling of k-space was re-gridded to a rectangular matrix with an approximate in-plane resolution of 3.6mm. The sequence used 4 control-label pairs. CBF maps were computed from the mean perfusion weighted difference image derived from the four control-label pairs, by scaling the difference image against a proton density image acquired at the end of the sequence, using identical readout parameters. This computation was done according to the formula suggested in the recent ASL consensus article [9]. The sequence used four background suppression pulses to minimise static tissue signal at the time of image acquisition. The entire acquisition time of the 3D-pCASL sequence was 6:20 minutes for each run.

We also acquired a three-dimensional sagittal high-spatial-resolution Inversion Recovery Spoiled Gradient Echo (IR-SPGR) T1-weighted scan (TE=3.016ms, TR=7.31ms, TI=400ms, FoV=270mm). The final resolution of the image was 1.1 x 1.1 x 1.2mm.

Image Processing

ASL data were preprocessed using the Automatic Software for ASL Processing (ASAP) 2.0 toolbox [10] running in Statistical Parametric Mapping version 12 (SPM12; https://www.fil.ion.ucl.ac.uk/spm/) and Matlab R2017a using the following procedure [11,12] for each run: (1) the origin of CBF and 3D T1-weighted images was realigned; (2) 3D T1-weighted images were segmented to generate a binary mask including only brain tissues; (3) CBF maps were co-registered to the corresponding 3D T1-weighted images; (4) extra-cerebral signal was removed from the CBF map by multiplication of the "brain only" binary mask, obtained in step 2, with the CBF map in the space of the T1 image; and (5) T1-weighted scans and skull-stripped CBF maps were spatially normalised to MNI avg152 standard space. For each subject, a mean (average) CBF map was obtained from the two preprocessed CBF maps (runs 1 and 2) using the "imcalc" function in SPM12. Finally, CBF maps were spatially smoothed using a 6mm Gaussian smoothing kernel [11].

Statistical Analysis

Pre-scan anxiety scores

For pre-scan anxiety (STAI [13]) scores, across all subjects (N=29) combined, 4 individual items out of a total of 580 (20-item scale x 29 subjects) were missing. These 4 items were therefore imputed using next-observation-carried-backward [14].

Repeated-measures analyses of covariance (RM-ANCOVAs) in STATA

Because pre-scan anxiety scores and global grey matter CBF were scan-level covariates, the data were prepared in STATA in long format, which allows specification of a covariate value for each level of the repeated factor (drug: oxytocin vs placebo). This procedure allows controlling for time-varying covariates within a paired-samples (here within-subject) design.

Exploratory/supplemental analyses

For runs with significant results at the level of the whole hippocampus (run 1 only), we used analogous procedures to those described for the whole hippocampal ROI to extract mean rCBF values for each hippocampal subregion individually. Separate masks for left CA1, CA2, CA3, dentate gyrus, and subiculum were anatomically defined using the cytoarchitectonic probabilistic atlas [15] in the SPM Anatomy [16] toolbox (see Figure 3A in the main text). Mean rCBF values for each subregion ROI were extracted and log transformed due to deviations from distributional assumptions for parametric tests. These values were then entered into RM-ANCOVAs in STATA. No multiplicity correction was applied as subregion analyses were exploratory.

Results

Sensitivity analyses - hippocampal rCBF

Eight participants were taking antidepressants, one of which was also taking benzodiazepines. To ensure that inclusion of these cases was not unduly influencing our results, we repeated the primary analyses (left hippocampal ROI rCBF analyses) after exclusion of these 8 cases. Here, we found that compared to placebo, oxytocin administration was associated with increased hippocampal rCBF in run 1 ($F(1,19)=11.37$, $p=.0032$), run 2 ($F(1,19)=8.12$, $p=.010$) and the mean of runs ($F(1,19)=10.64$, $p=.0041$), all of which survived Hochberg multiplicity correction. After controlling for global signal effects, oxytocin administration was associated with increased hippocampal rCBF in run 1 ($F(1,18)=6.10$, $p=.024$), however, this result did not survive multiplicity correction. The effects were also no longer evident in run 2 ($F(1,18)=1.01$, $p=.33$) or in the mean of runs ($F(1,18)=3.68$, $p=.071$).

Whole-brain results, adjusted for global effects

Results adjusted for global effects are reported in Table S1 below. After controlling for global CBF effects, in run 1, oxytocin administration was associated with decreased perfusion in a cluster spanning the bilateral anterior cingulate and frontal cortices ($p_{FWE}<.05$), a separate cluster in the right inferior frontal gyrus ($p_{FWE}<.05$), and increased CBF in the left visual cortex ($p_{FWE}<.05$) (and see Discussion below). There were no suprathreshold whole-brain effects for either contrast in run 2 or in the mean of runs.

Tables & Figures

Table S1. Effects of oxytocin vs placebo on whole-brain CBF (with adjustment for global CBF effects)

Cluster Description	Hemis-phere	*k*	$P_{(FWE\text{-}corr)}$	Peak coordinates			Peak Description
				x	y	z	
Run 1, Oxytocin > Placebo							
Left visual cortex, calcarine gyrus; right visual cortex	Left	620	<.05	-12	-72	16	Primary visual cortex (V1, V2)
				-6	-80	28	Visual association cortex (dorsal V3)
				-14	-72	-2	Visual association cortex (ventral V3)
Run 1, Placebo > Oxytocin							
Left anterior cingulate cortex/gyrus, frontal pole, superior medial frontal gyrus; bilateral mid orbital gyrus	Left	581	<.05	-2	36	26	Anterior cingulate cortex
				6	48	-12	Frontal pole
				-2	28	42	Superior medial frontal gyrus
Right inferior frontal gyrus	Right	383	<.05	50	14	22	Inferior frontal gyrus (pars opercularis)
				48	28	22	Inferior frontal gyrus (pars triangularis)
				60	4	12	Rolandic operculum
Run 2, Oxytocin > Placebo							
None							
Run 2, Placebo > Oxytocin							
None							
Mean of runs, Oxytocin > Placebo							
None							
Mean of runs, Placebo > Oxytocin							
None							

k, number of voxels in the cluster; P_{FWE}, FWE-corrected p-value.

Figure S1. rCBF Effects in Left Hippocampus. Bar charts showing mean hippocampal rCBF in the oxytocin and placebo conditions in runs 1 and 2 without adjustment for global effects.

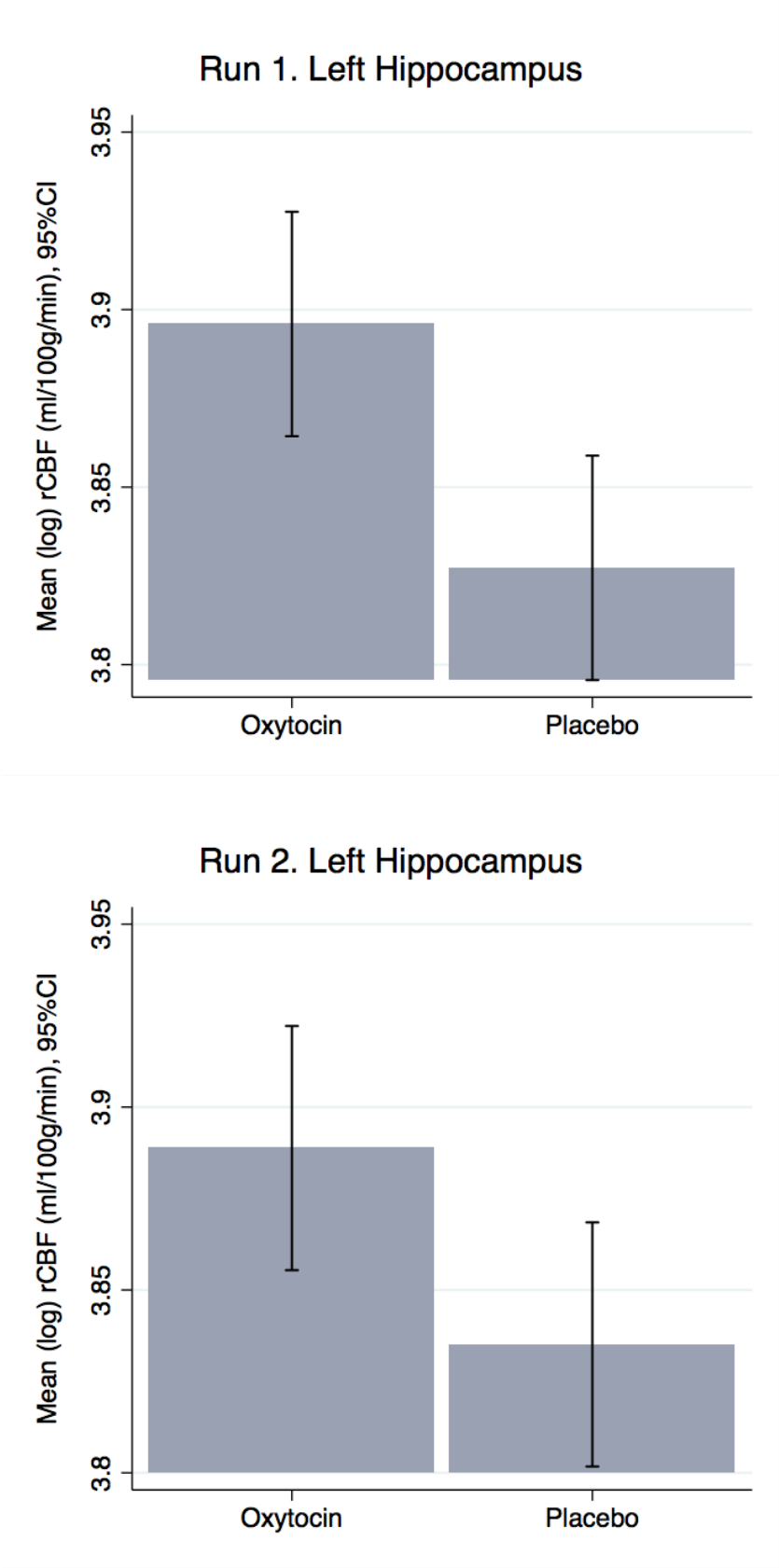
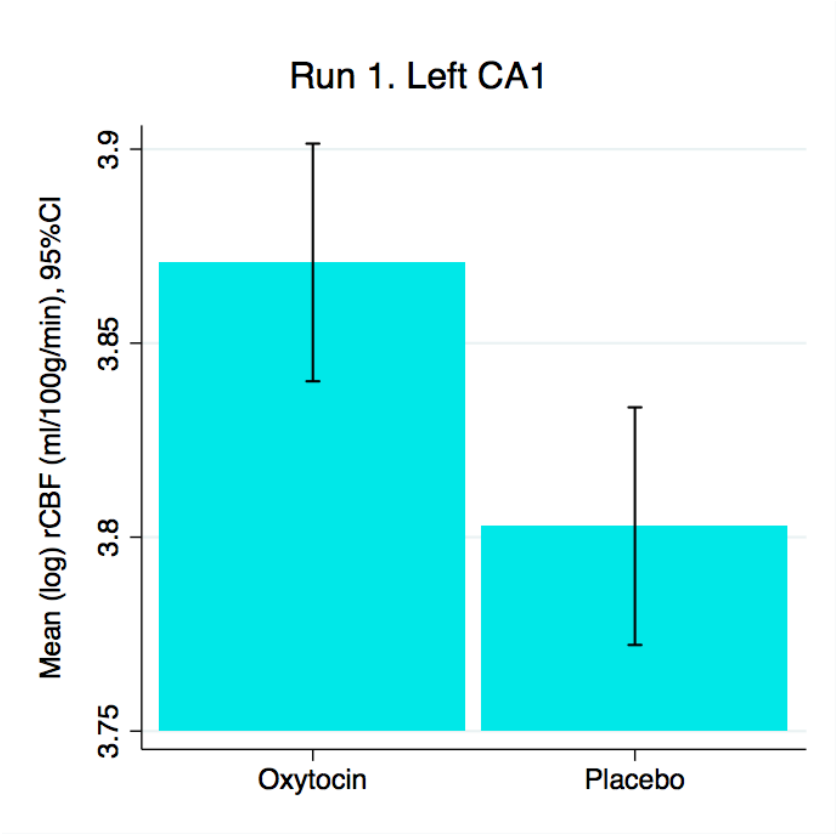
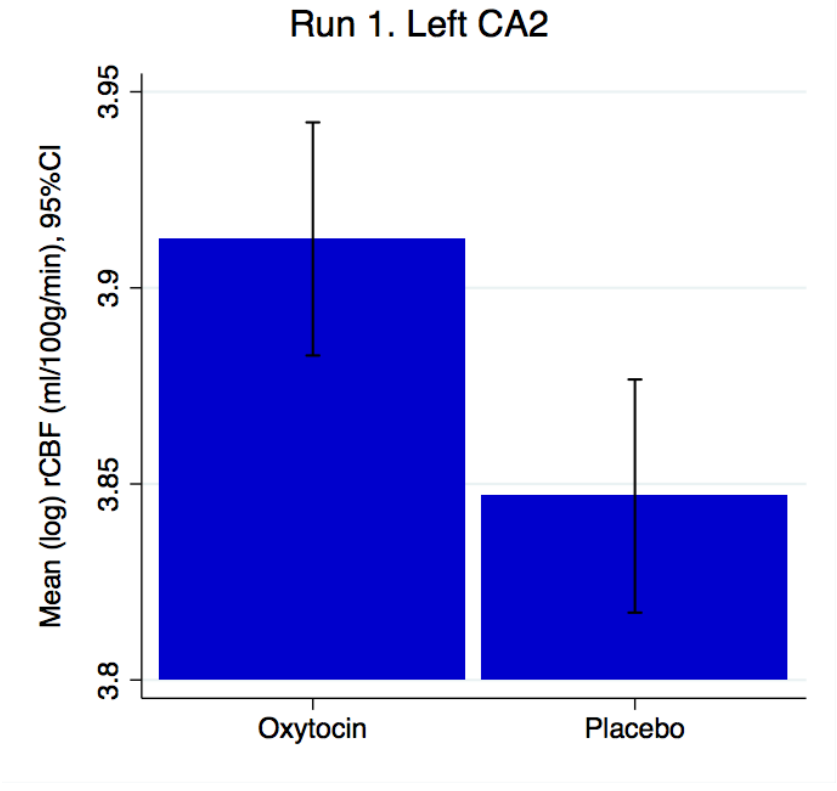
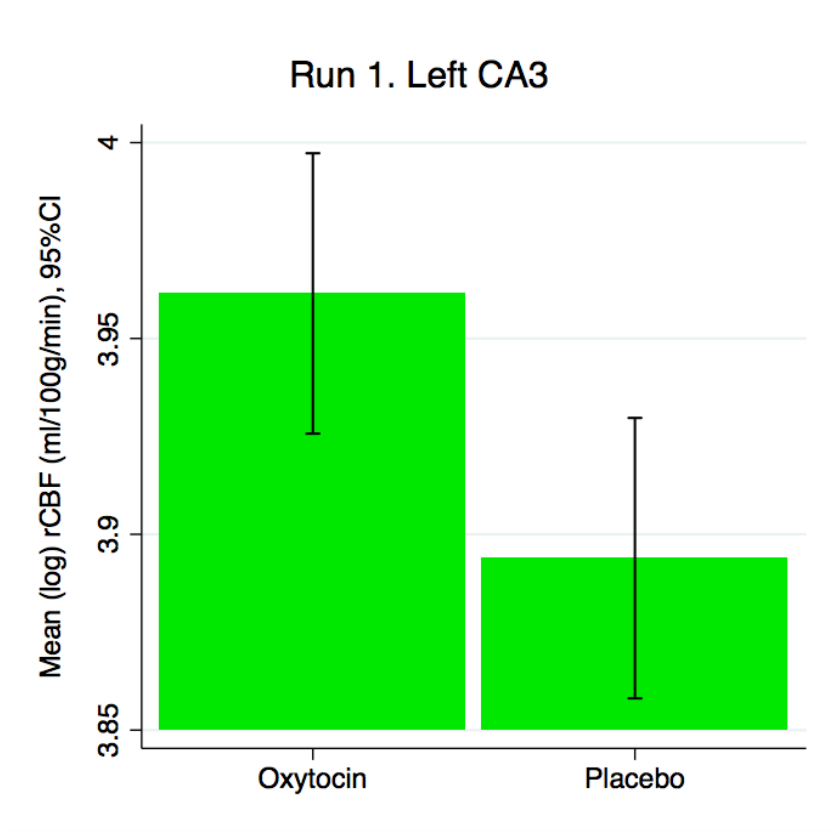
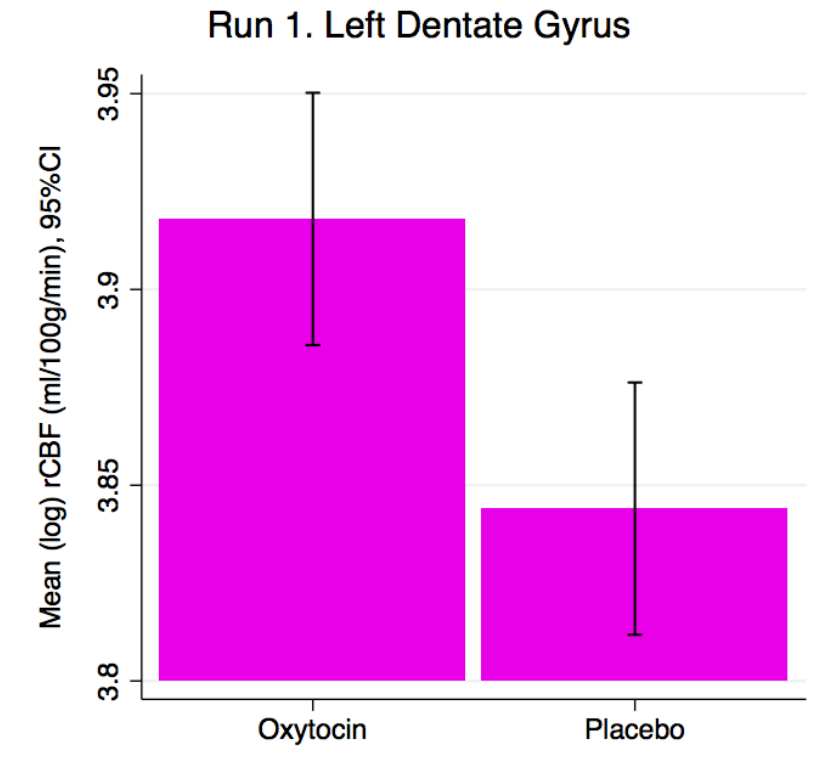


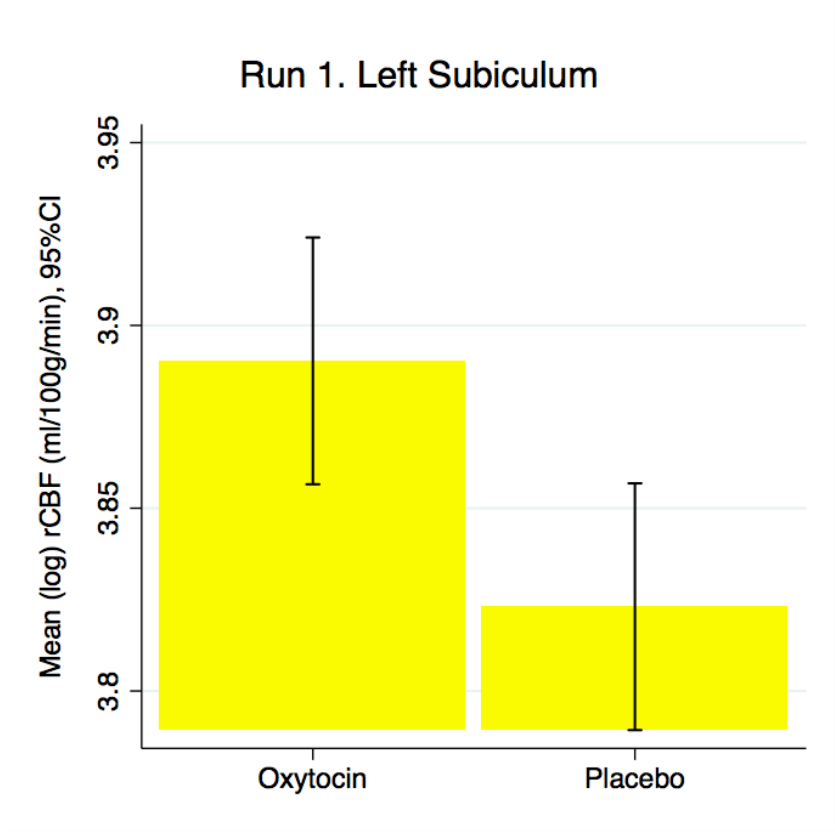
Figure S2. rCBF in Left Hippocampal Subregions. Bar charts showing mean hippocampal subregion rCBF in the oxytocin and placebo conditions in run 1 without adjustment for global effects.






Run 1. Left CA3
Mean (log) rCBF (ml/100g/min), 95%CI
4
3.95
3.9
3.85
Oxytocin
Placebo


Run 1. Left Dentate Gyrus
Mean (log) rCBF (ml/100g/min), 95%CI
3.95
3.9
3.85
3.8
Oxytocin
Placebo


Run 1. Left Subiculum
Mean (log) rCBF (ml/100g/min), 95%CI
3.95
3.9
3.85
3.8
Oxytocin
Placebo

Discussion

Whole-brain results corrected for global signal effects

While the temporal/cerebellar whole-brain results (as reported in the main text) were not observed after inclusion of global signal as a nuisance covariate, there were a number of clusters that were significant, including increased perfusion in visual cortex, but perhaps more surprisingly, we also observed decreased perfusion across the anterior cingulate cortex (ACC) and inferior frontal gyrus (IFG) in the oxytocin vs placebo condition. Postmortem immunohistochemical studies have found oxytocin receptors and fibres in the ACC [17], and numerous associations between oxytocin receptor gene polymorphisms and ACC volume [18] as well as ACC/IFG functional activation have been reported [19,20]. Our findings are also consistent with the attenuated engagement observed in these regions in functional MRI studies [21] after oxytocin. However, this contrasts with the previous oxytocin study in healthy males, which found increased perfusion in the ACC and IFG in response to oxytocin [7], but it should be noted that this previous study [7] was a between-group comparison. These differential effects of oxytocin on the ACC/IFG might also be explained by differences in baseline rCBF in prefrontal regions in CHR-P individuals relative to controls, as have recently been reported [22].

Further hippocampal subregions

While we were *a priori* interested in CA1, which we have discussed along with the dentate gyrus in the main text discussion, we also found increased perfusion after oxytocin in the subiculum and CA2 subregion. As noted in the introduction, converging lines of evidence implicate CA1 hypermetabolism/hyperperfusion as a driver of the pathophysiological processes underlying the onset of psychosis [2]. However, research has shown that longitudinally, this CA1-originating dysfunction appears to progressively spread to the subiculum (and then to extra-hippocampal regions such as the frontal cortex) at the time of onset of psychosis in CHR-P individuals who transition vs those who do not [23]. This is combined with volumetric and morphometric shape changes occurring from the time of a CHR-P state to the onset of frank psychosis, again with the greatest changes observed in the subiculum (and CA1)[23]. Another study found selectively reduced subiculum volumes and worse verbal recall scores in familial high-risk individuals vs low genetic risk controls, with recall performance significantly correlated with subicular volume [24]. In terms of the CA2 subregion, studies have reported reduced CA2/3 volumes in non-remitted CHR-P individuals relative to healthy controls [25]. Reduced CA2/3 volumes [26,27] and greater longitudinal reductions in CA2/3 volume (which correlated with worsening general psychopathology scores over time) are also seen in patients with schizophrenia relative to healthy controls [28]. Intriguingly, oxytocin receptors are abundantly expressed in CA2 (at least in rodents) [29] and oxytocin receptor knock-out studies show that oxytocin signalling in CA2 is crucial for the persistence of long-term social recognition memory [30].

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6. OXYTOCIN – 1H-MRS STUDY

6.1. PAPER 4 – OXYTOCIN 1H-MRS

Davies C, Rutigliano G, De Micheli A, Stone JM, Ramella-Cravaro V, Provenzani U, et al (2019). Neurochemical effects of oxytocin in people at clinical high risk for psychosis. *Eur Neuropsychopharm*. https://doi.org/10.1016/j.euroneuro.2019.03.008

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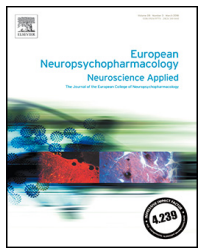

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Neurochemical effects of oxytocin in people at clinical high risk for psychosis

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KEYWORDS
Glutamate;
Oxytocin;
Magnetic resonance spectroscopy;
Psychosis risk;
Schizophrenia;
Neuroimaging

Abstract
Alterations in neurochemical metabolites are thought to play a role in the pathophysiology of psychosis onset. Oxytocin, a neuropeptide with prosocial and anxiolytic properties, modulates glutamate neurotransmission in preclinical models but its neurochemical effects in people at high risk for psychosis are unknown. We used proton magnetic resonance spectroscopy (^{1}H-MRS) to examine the effects of intranasal oxytocin on glutamate and other metabolites in people at Clinical High Risk for Psychosis (CHR-P) in a double-blind, placebo-controlled, crossover design. 30 CHR-P males were studied on two occasions, once after 40IU intranasal oxytocin and once after placebo. The effects of oxytocin on the concentration of glutamate, glutamate+glutamine and other metabolites (choline, N-acetylaspartate, myo-inositol) scaled to creatine were examined in the left thalamus, anterior cingulate cortex (ACC) and left hippocampus, starting approximately 75, 84 and 93 min post-dosing, respectively. Relative to placebo, administration of oxytocin was associated with an increase in choline levels in the ACC (p=.008, Cohen's d = 0.54). There were no other significant effects on metabolite concentrations (all p>.05). Our findings suggest that, at ~75-99 min post-dosing, a single dose of intranasal oxytocin does not alter levels of neurochemical metabolites in the thalamus, ACC, or hippocampus in those at CHR-P, aside from potential effects on choline in the ACC.

1. Introduction

The onset of psychosis is usually preceded by attenuated psychotic symptoms (Fusar-Poli et al., 2016b) and a clinical presentation that can include social, cognitive and functional impairment (Fusar-Poli et al., 2012a, 2013a, 2015). Such individuals are said to be at Clinical High-Risk for Psychosis (CHR-P) (Fusar-Poli, 2017) and have approximately 20% risk of developing a first-episode psychosis over the following two years (Fusar-Poli et al., 2016a). The neurobiological mechanisms underlying psychosis onset remain incompletely understood, but alterations in regional brain structure, function and neurochemical composition have been observed in CHR-P samples (Allen et al., 2016; Bartholomeusz et al., 2017; Fusar-Poli et al., 2012b; Modinos et al., 2018a).

In particular, accumulating evidence suggests that the pathophysiological processes underlying psychosis onset may be driven by dysregulated glutamate neurotransmission (Lieberman et al., 2018), with hypofunction of N-methyl-D-aspartate receptors (NMDAR) on γ-aminobutyric acid (GABA)-ergic interneurons leading to disinhibition of pyramidal cells, and via polysynaptic projection pathways from the hippocampus to the midbrain/striatum, to midbrain hyperdopaminergia (Fig. 1) (Lisman et al., 2008; Modinos et al., 2015). Glutamate is an ubiquitous neurotransmitter with excitotoxic potential (Farber et al., 1995), and animal models have demonstrated that excess extracellular glutamate is sufficient to induce hippocampal hypermetabolism and volume loss (Lieberman et al., 2018; Schobel et al., 2013) comparable to that observed as the CHR-P state progresses to psychosis (Allen et al., 2016; Ho et al., 2017; Schobel et al., 2013). Novel pharmacotherapies that can modulate the glutamate system—or associated neural circuits—have therefore become a target for drug development (Lieberman et al., 2018; Millan et al., 2016).

In humans, metabolite levels can be quantified in vivo using proton magnetic resonance spectroscopy (^{1}H-MRS) (Provencher, 1993). Using this technique, numerous (but not all (Merritt et al., 2016)) studies have reported alterations in glutamate (and/or glutamate plus glutamine; Glx) levels in people at high risk of psychosis vs. controls, particularly in the hippocampus (Bloemen et al., 2011; Shakory et al., 2018), frontal (Merritt et al., 2016) or anterior cingulate cortex (ACC)(Stone et al., 2009; Tibbo et al., 2004) and thalamus (Stone et al., 2009). Moreover, within CHR-P samples, altered metabolite levels at baseline appear related to clinical outcomes; thalamic glutamate is lower at baseline in those who do not remit by follow up (Egerton et al., 2014), and baseline hippocampal glutamate is elevated in those who transition (vs. those who do not), and in those with poor (vs. good) functional outcomes (Bossong et al., 2018). There is also evidence that neurochemical alterations in CHR-P are not restricted to glutamate or Glx; higher levels of hippocampal myo-inositol at first presentation have been reported in CHR-P patients with poor functional and clinical outcomes (with effect sizes surpassing that of glutamate), which suggests that hippocampal integrity may be compromised more generally (Bossong et al., 2018). Metabolite alterations have also been observed in other brain regions in CHR-P and other (i.e., familial) high-risk groups, such as reduced N-acetylaspartate in the thalamus (Stone et al., 2009; Tandon et al., 2013; Yoo et al., 2009), ACC (Capizzano et al., 2011; Jessen et al., 2006), and caudate (Keshavan et al., 2009)—although increases are also observed in the caudate (de la Fuente-Sandoval et al., 2011). Increased Glx has been reported in the thalamus and caudate, as well as increased (de la Fuente-Sandoval et al., 2016) or decreased (Menschikov et al., 2016) medial prefrontal GABA—although differences are not always found (Modinos et al., 2018b; Wang et al., 2016). Finally, other studies have reported increased choline in the ACC (Tandon et al., 2013), prefrontal cortex (Wood et al., 2003) and hippocampus (Capizzano et al., 2011) in populations at risk for psychosis.

Prevention of psychosis in patients at CHR-P and amelioration of symptoms are clinical priorities. However, there

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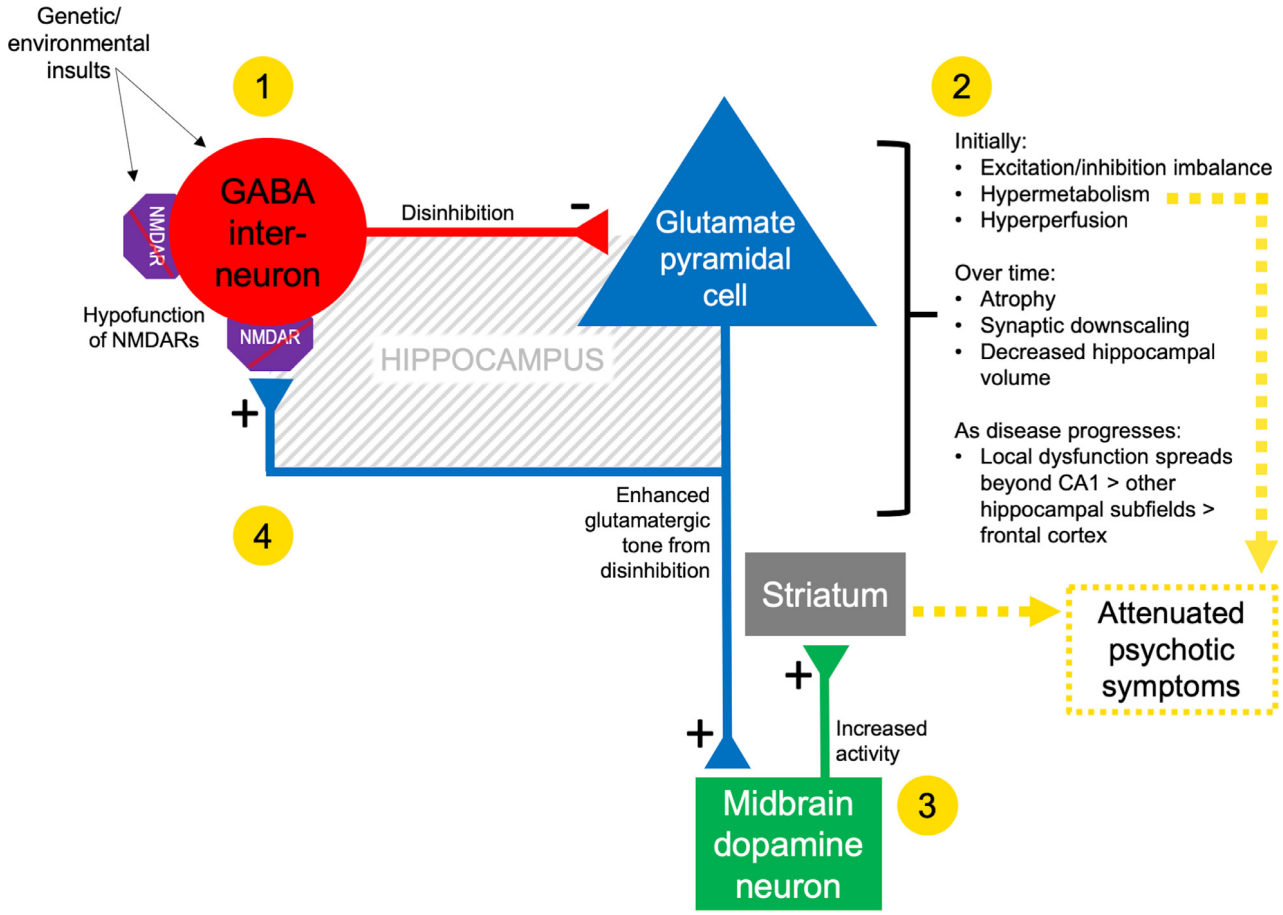

Fig. 1 *Simplified schematic of proposed neural circuit mechanisms of glutamatergic dysfunction and CHR-P pathophysiology*. In (1), low glutamate signal/input from hypofunctioning NMDARs (akin to 'faulty homeostatic sensors') leads GABAergic interneurons to homeostatically increase excitation by reducing inhibition (disinhibition) of glutamatergic pyramidal cells. However, by disinhibiting pyramidal cells (and thus increasing glutamate signalling) in this dysfunctional neural environment, the potential homeostatic adaptation becomes allostatic (2). In (3), enhanced excitation leads to an overdrive in the responsivity of midbrain dopamine neurons which project to the associative striatum (note that the connection between hippocampal pyramidal cells and midbrain dopamine neurons is presented as monosynaptic but is actually polysynaptic via the ventral striatum and ventral pallidum). Completing the (simplified) circuit, local glutamatergic tone is increased in (4) but is not detected as such by hypofunctioning NMDARs on GABAergic interneurons. Figure reproduced and adapted with permission (CCBY 4.0)[1] from (Davies et al., 2019). For original diagrams and discussion of evidence for this proposed circuit, see (Krystal et al., 2017; Krystal and Anticevic, 2015; Lieberman et al., 2018; Lisman et al., 2008; Modinos et al., 2015). *Abbreviations:* Glu, glutamate; NMDAR, N-methyl-D-aspartate receptor; CA1, Cornu Ammonis 1.

are currently no licensed pharmacological treatments for this patient group, which remains an unmet clinical need (Davies et al., 2018a, b). One potential novel treatment is the neuropeptide oxytocin, which is currently under investigation in relation to a number of neuropsychiatric disorders characterised by social dysfunction, such as autism spectrum disorder and schizophrenia. Oxytocin is a key modulator of social and emotional processes and possesses anxiolytic, prosocial, and potential anticonvulsant properties (Domes et al., 2007; Guastella et al., 2008; Kanat et al., 2015; Kirsch, 2005; Meyer-Lindenberg et al., 2011). Intranasal oxytocin is also safe and has a benign side effect profile (MacDonald et al., 2011), which is particularly important in CHR-P groups because many will not go on to develop psychosis. The effects of oxytocin on brain metabolites are not completely clear, but available evidence implicates some of the metabolites that have been found to be abnormal in CHR-P individuals: glutamate, Glx, N-acetylaspartate, choline and GABA. Specifically, in a previous double-blind, placebo-controlled crossover trial of a single acute dose of 24IU intranasal oxytocin in people with autism spectrum disorder, oxytocin's normalisation of brain activation during a social cognition task and improvement in social-communication symptoms was found to be related to its effects on N-acetylaspartate levels (Aoki et al., 2015). Choline levels in the ACC also appeared to be decreased by oxytocin, albeit at a relaxed significance threshold (p=.02,

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uncorrected for multiple comparisons; see supplementary material in (Aoki et al., 2015)). In a separate double-blind, placebo-controlled, crossover trial of 48IU/day oxytocin, which was administered repeatedly over 6 weeks in people with autism spectrum disorder, oxytocin significantly reduced levels of Glx and N-acetylaspartate in the medial prefrontal cortex/ACC relative to placebo (Benner et al., 2018). This prior work demonstrates that exogenously administered oxytocin can alter levels of metabolites as measured by ^{1}H-MRS. Evidence for effects of oxytocin on glutamate and GABA comes from preclinical studies. For instance, oxytocin dose-dependently restores social and sensorimotor gating deficits in NMDAR antagonist-treated rats (Feifel and Reza, 1999; Lee et al., 2005). Mice lacking the oxytocin receptor (through gene knock-out) are more vulnerable to NMDAR antagonist-induced sensorimotor gating deficits (but not those induced by amphetamine or apomorphine), which suggests that it is the glutamatergic (rather than dopaminergic) component of sensorimotor gating that is protected by oxytocin (Caldwell et al., 2009). In mice, oxytocin attenuates methamphetamine-induced changes in glutamatergic neurotransmission in prefrontal cortex and hippocampus via regulation of NMDAR subunit (NR1) and glutamate transporter (GLT1) expression (Qi et al., 2012). Furthermore, in animals, oxytocin enhances the signal-to-noise ratio of pyramidal cell firing by targeting fast-spiking GABAergic interneuron function (Owen et al., 2013; Zaninetti and Raggenbass, 2000)—part of the neural circuit implicated in psychosis onset (Lieberman et al., 2018; Lisman et al., 2008; Modinos et al., 2015). When this circuit is acutely manipulated in humans, for example, by inducing NMDAR hypofunction using NMDAR antagonists, or by reducing glutamate release (e.g., using n-acetyl-cysteine), increases and decreases (respectively) in the concentrations of regional glutamate/Glx levels as measured by ^{1}H-MRS can be observed (Kegeles et al., 2014; Kraguljac et al., 2017; McQueen et al., 2018; Schmaal et al., 2012). This demonstrates that the acute modulation of microcircuit function can alter metabolite concentrations, and that this can be observed experimentally using ^{1}H-MRS.

Despite these initial findings, no studies have yet examined the effects of intranasal oxytocin on neurochemical metabolites in CHR-P individuals, which would further our understanding of its neurophysiological mechanism of action and potential disease-engaging effects. To fill this gap in knowledge, we investigated the effects of intranasal oxytocin on several neurochemical metabolites that are thought to be altered in CHR-P individuals in a double-blind, oxytocin vs. placebo single-dose challenge crossover ^{1}H-MRS study. In line with previous findings in CHR-P individuals, our primary aim was to assess the effects of oxytocin on levels of glutamate, and glutamate plus glutamine (Glx), in the left hippocampus, ACC and left thalamus (Bloemen et al., 2011; Shakory et al., 2018; Stone et al., 2009; Tibbo et al., 2004). In view of recent literature suggesting that N-acetylaspartate, myo-inositol and choline may be altered in populations at risk of psychosis (Bossong et al., 2018; Tandon et al., 2013), and that oxytocin's effects may involve several neurochemical pathways (Aoki et al., 2015; Benner et al., 2018; Ninan, 2011; Qi et al., 2012), our secondary aim was to examine the effects of oxytocin on these metabolite levels.

2. Experimental procedures

2.1. Participants

The study received National Research Ethics Service approval (14/LO/1692) and all subjects gave written informed consent. Using data from a previous ^{1}H-MRS study (Stone et al., 2009) we conducted a power calculation using G*Power 3, which indicated that a sample size of 30 was sufficient to detect a medium within-subject effect size (d_z=0.53), when alpha = 0.05 and power = 80%. Accordingly, 30 male, help-seeking CHR-P individuals aged 18–35 were recruited from two specialist early detection services—the Outreach and Support in South London (OASIS) (Fusar-Poli et al., 2013b) and Tower Hamlets Early Detection Service (THEDS). A CHR-P status was determined using the Comprehensive Assessment of At-Risk Mental States (CAARMS) 12/2006 criteria (Yung et al., 2005). The CHR-P state is heterogeneous (Fusar-Poli et al., 2016a) because subjects can meet one or more of the following subgroup criteria: (a) attenuated psychotic symptoms, (b) brief limited intermittent psychotic symptoms (BLIPS, psychotic episode lasting <1 week, remitting without treatment), or (c) either schizotypal personality disorder or first-degree relative with psychosis (Yung et al., 2005), all coupled with functional decline. Individuals were excluded if there was a history of previous psychotic disorder (with the exception of BLIPS (Fusar-Poli et al., 2016), some of whom may meet Acute and Transient Psychotic Disorder criteria (Fusar-Poli et al., 2017; Fusar-Poli et al., 2018)) or manic episode, exposure to antipsychotics, neurological disorder or current substance-use disorder, estimated IQ <70, acute intoxication on the day of scanning, and any contraindications to magnetic resonance imaging (MRI) or intranasal oxytocin or placebo.

2.2. Procedure

We used a double-blind, 40IU intranasal oxytocin vs. placebo single-dose challenge in a crossover design (one-week wash out). During each challenge, subjects underwent an MRI scan which started at 11:30 AM to minimise potential effects of diurnal variation in oxytocin or vasopressin (Paloyelis et al., 2016). Prior to the first scan, subjects completed a computerised Reading the Mind in the Eyes (RMET) task (Baron-Cohen et al., 2001), which indexes mentalising (theory of mind) ability, for use in correlation analyses. The RMET requires participants to match the mental state of a person (as shown in a photograph of the eye region only) with one of four possible mental state words. Higher scores (out of a maximum of 36) index better mentalising ability. For descriptive purposes, we also collected information on baseline anxiety (State-Trait Anxiety Inventory [STAI]), medication history, use of alcohol (Alcohol Use Disorders Identification Test [AUDIT]), tobacco and cannabis, and functioning using the Global Functioning (GF) Role and Social scales (Cornblatt et al., 2007). Intranasal administration followed recommended guidelines and a protocol adopted by a previous study conducted at our institute (Paloyelis et al., 2016). Briefly, participants self-administered one puff (4IU) of intranasal oxytocin or matched placebo every 30 s, alternating between nostrils, until 40IU had been administered (Supplementary Material). Although not presented here, the MRI scan included arterial spin labelling, a functional MRI (fMRI) task, various structural scans and resting state fMRI, followed by the three ^{1}H-MRS sequences (detailed below).

2.3. Magnetic resonance imaging

All scans were conducted on a General Electric Discovery MR750 3 Tesla system (General Electric, Chicago, USA) using a 32-

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channel head coil. A three-dimensional sagittal high-spatial-resolution Inversion Recovery Spoiled Gradient Echo (IR-SPGR) T1-weighted scan (TE=3.016 ms; TR=7.31 ms; TI=400 ms; voxel size=1.1 × 1.1 × 1.2 mm^3) was acquired for voxel planning and calculation of ^{1}H-MRS voxel tissue content. ^{1}H-MRS spectra were acquired in the left hippocampus, ACC, and left thalamus (Fig. 2) using conventional Point-Resolved Spectroscopy acquisition (PRESS; TR = 3000 ms; TE = 30 ms; 96 averages) in three separate 6-min scans. We employed the standard GE PROBE (Proton Brain Examination) sequence, which uses a standardised chemically selective suppression (CHESS) water suppression routine. Unsuppressed water reference spectra (16 averages) were also acquired as part of the standard acquisition for subsequent eddy current correction and water scaling. Shimming was optimised, with auto-prescan performed twice before each scan. The structural T1-weighted scan was used to plan the voxel placement as per standardised protocols, with voxel sizes of (right-left, anterior-posterior, superior-inferior) 20 × 20 × 15 mm^3 in the hippocampus, 20 × 20 × 20 mm^3 in the ACC, and 15 × 20 × 20 mm^3 in the thalamus. The mean ± SD of the time from dosing offset (end of intranasal administration) for each region was: thalamus (75 ± 4 min), ACC (84 ± 5 min), and hippocampus (93 ± 6 min). These equate to approximate (mean) post-dosing sampling periods of: thalamus (75–81 min), ACC (84–90 min), and hippocampus (93–99 min).

2.4. Data processing

Spectra were analysed using LCModel (Provencher, 1993) version 6.3-1L using the standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, and taurine) acquired at the same field strength (3 T), localisation sequence (PRESS), and echo time (30 ms). Model metabolites and concentrations used in the basis set are fully detailed in the LCModel manual (http://s-provencher.com/pub/LCModel/manual/manual.pdf). Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds [CRLB] of >20% as reported by LCModel) were excluded from further analysis. Spectral quality was further assessed using signal-to-noise ratio (SNR) and spectral linewidths (full width at half-maximum; FWHM).

Our primary analysis used metabolite levels scaled to creatine. However, because creatine may be influenced by the experimental condition (oxytocin vs. placebo), we also report water-scaled metabolite levels corrected for voxel tissue content. To calculate and correct for ^{1}H-MRS voxel tissue content, an in-house script was used to (a) segment the T1-weighted structural images into grey matter, white matter, and cerebrospinal fluid (CSF) using Statistical Parametric Mapping 12 (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK) running in Matlab R2017a, (b) locate and map the coordinates of each voxel to the segmented T1 images, and (c) provide the tissue content proportions. Metabolite values were corrected for voxel tissue content using the formula: $M_{corr} = M \times ([GM \times 1.21] + WM + [CSF \times 1.55]) / (WM+GM)$, where M is the uncorrected metabolite value and GM/WM/CSF are proportions of grey matter, white matter and CSF, respectively. The formula assumes a CSF water concentration of 55,556 mol/m^3 and the LCModel default brain water concentration of 35,880 mol/m^3 (Gasparovic et al., 2006; Kreis et al., 1993). Apart from assuming T_2 = 80 ms for tissue water, no corrections were applied for metabolite and water relaxation times.

2.5. Statistical analysis

Statistical analyses were performed in SPSS version 24 (IBM Corp., Armonk, NY). Paired t-tests were used to examine differences in data quality (i.e., in linewidths, signal-to-noise ratio, CRLB, and voxel tissue proportions) between conditions. The effects of oxytocin vs. placebo on regional brain ^{1}H-MRS metabolite levels were assessed using paired t-tests. Our primary hypothesis tested for effects on glutamate and Glx scaled to creatine, and our secondary hypothesis tested for effects on other metabolites (myo-inositol, N-acetylaspartate and choline) scaled to creatine. Choline (or "total choline") was the sum of glycerophosphocholine and phosphocholine. The alpha level was Bonferroni corrected (thresholded $p<.05/5$ metabolites = $p<.01$, two-tailed) for multiple comparisons per region. For both primary and secondary analyses, boxplots were used to identify potential outliers and paired t-tests were repeated after their exclusion in sensitivity analyses. We also repeated the primary and secondary analyses after excluding those participants taking antidepressants ($N=8$) or benzodiazepines ($N=1$). The same procedures as detailed above were used in the analysis of water-scaled metabolite levels corrected for voxel tissue content (which also included analysis of creatine itself), which we conducted to ensure that our results were not driven by the scaling to creatine. Exploratory Spearman's correlations were then used to examine whether the effects of oxytocin on the primary outcome (change in glutamate and Glx levels—scaled to creatine—in the hippocampus, ACC and thalamus in the oxytocin vs. placebo condition) were predicted by the baseline level of behavioural measures that characterise CHR-P patients. These included attenuated positive psychotic symptoms (sum of severity scores for items P1-P4 of the CAARMS) and social cognition, measured through the Reading the Mind in the Eyes task (RMET) scores. Since these correlations were exploratory in nature they were not corrected for multiple comparisons. Finally, given some attrition in study participation, we updated our power calculation for the primary hypotheses (glutamate and Glx) to assess the actual power of our analyses (see Supplementary Material).

3. Results

3.1. Sample characteristics

Demographic and clinical characteristics of the sample are presented in Table 1. One subject was removed due to protocol violations, and one further subject did not complete the scanning session due to experiencing attenuated psychotic symptoms (at ~75min during the placebo condition), leaving a sample of $N=28$ for the ACC and thalamus. Two further subjects did not complete the hippocampal ^{1}H-MRS scan, leaving $N=26$ for this region. No adverse side effects of oxytocin were clinically observed.

3.2. ^{1}H-MRS data quality

Representative spectra for all regions (left hippocampus, ACC, and left thalamus) are provided in Fig. 2. Spectra were of good quality in all regions—no data were excluded due to Cramer-Rao minimum variance bounds >20% and no significant differences in spectral quality or voxel tissue content were observed between oxytocin and placebo conditions (Table 2).

3.3. Effects of oxytocin on glutamate and Glx levels

The analysis of the primary outcome revealed no significant effects of oxytocin vs. placebo on glutamate or Glx scaled

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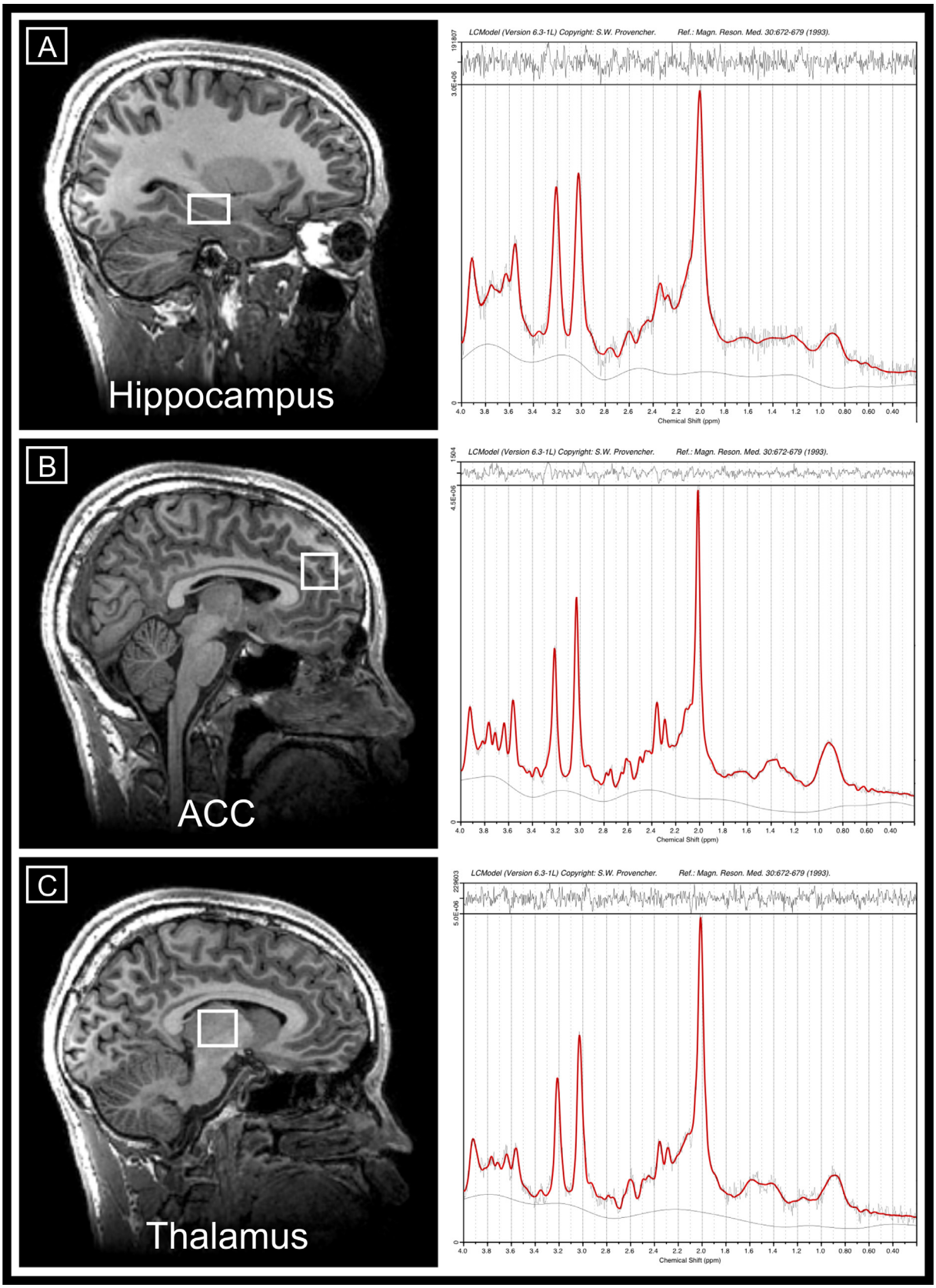

Fig. 2 Example ^{1}H-MRS voxel positioning and spectra in (A) left hippocampus, (B) anterior cingulate cortex, and (C) left thalamus.

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Table 1 Participant demographic and clinical characteristics.

	Variable	Total sample ($N = 30$)
Demographic	Age, years; mean (SD)	23.2 (4.7)
	Age range, years	18-35
	Sex, male/female	30/0
	Ethnicity (White/Black/Asian/Mixed)	16/6/4/4
	Handedness, right/left	26/4
	Education, years; mean (SD)	13.2 (1.9)
Clinical	CHR-P Subtype[a] (BLIPS/APS/GRD)	6/23/1
	CAARMS attenuated positive symptoms;[b] mean (SD)	11.7 (3.3)
	Baseline anxiety score;[c] mean (SD)	35.6 (8.7)
	GF social score; mean (SD)	6.8 (1.5)
	GF role score; mean (SD)	7.0 (1.7)
	RMET score;[d] mean (SD)	24.7 (5.6)
	Current antidepressant medication (yes/no)	8/22
	Current antipsychotic medication (yes/no)	0/30
	Current benzodiazepine medication (yes/no)	1/29
Substance use	Current smoker (yes/no)	17/13
	Cigarettes/day; mean (SD)	9.8 (6.0)
	Cannabis use;[e] median (range)	2 (0-4)
	Alcohol, AUDIT total; mean (SD)	7.2 (7.7)

[a] Comprehensive Assessment of At-Risk Mental States (CAARMS) subgroup, BLIPS - Brief Limited Intermittent Psychotic Symptoms; APS - Attenuated Psychotic Symptoms; GRD - Genetic Risk and Deterioration.
[b] Sum of the global (severity) ratings for positive subscale items (P1-P4) of the CAARMS.
[c] Mean of pre-scan anxiety scores across conditions as measured by the State Trait Anxiety Inventory (STAI), with 4 subjects missing 1/20 items that constitute the total score, these were thus imputed using next-observation-carried-backwards.
[d] Reading the Mind in the Eyes (RMET) social cognition task - two subjects did not complete the RMET, leaving $N = 28$.
[e] Cannabis use: 0 = never, 1 = experimental use (tried occasionally), 2 = occasional use (small quantities from time to time), 3 = moderate use (moderate quantities regularly / large amounts occasionally), 4 = severe use (frequently used large quantities, often to intoxication/debilitation). AUDIT - Alcohol Use Disorders Identification Test. CHR-P - Clinical High Risk for Psychosis. GF - Global Functioning (role and social) scale.

to creatine (nor in voxel tissue-corrected glutamate or Glx levels) in the hippocampus, ACC or thalamus (Table 3).

3.4. Effects of oxytocin on other metabolite levels

Analysis of the secondary outcomes revealed no significant effects on any other metabolites quantifiable from the ^{1}H-MRS spectra (myo-inositol, choline and N-acetylaspartate) in any region, with the exception of choline scaled to creatine in the ACC, which was significantly increased in the oxytocin (mean ± SD; 0.25 ± 0.02) relative to the placebo (mean ± SD; 0.24 ± 0.02) condition ($t(27)$=2.88, p=.008; Cohen's d = 0.54; Fig. 3) (Table 3). When using ACC choline levels corrected for voxel tissue content, we observed a numerical increase in the oxytocin (mean ± SD; 2.93 ± 0.36) relative to the placebo (mean ± SD; 2.77 ± 0.41) condition ($t(27)$=2.57, p=.02; Table 3), but this effect was not significant at the Bonferroni-corrected significance threshold of p<.01 (Table 3).

3.5. Sensitivity analysis

Removal of outliers made no material change to the results of any statistical test (Table 3). Removal of subjects taking antidepressants and benzodiazepines meant that choline scaled to creatine was now significantly increased in the thalamus in the oxytocin (mean ± SD; 0.30 ± 0.03) relative to the placebo (mean ± SD; 0.28 ± 0.03) condition ($t(20) = 3.11$, $p = .006$).

3.6. Exploratory correlations

3.6.1. Social cognition

One subject did not complete the Reading the Mind in the Eyes task (RMET), leaving $N = 25$ for the hippocampus, and $N = 27$ for the ACC and thalamus. RMET (theory of mind) scores were negatively associated with absolute change in glutamate scaled to creatine (rho = −0.546, $p = .005$) and Glx scaled to creatine (rho = −0.509, $p = .009$) in the hippocampus (Supplementary Table S1). That is, those with better theory of mind scores tended to have the biggest decreases in hippocampal glutamate after oxytocin, while those with lower scores (where oxytocin is presumed to have the greatest effect (Feeser et al., 2015; Spengler et al., 2017)) tended to have an increase in hippocampal glutamate after oxytocin (Supplementary Figure S1). There were no significant correlations between RMET scores and glutamate or Glx levels in the ACC or thalamus (all p>.05; Table S1). It should be noted that exploratory correlations were not corrected for multiple comparisons.

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Table 2 Spectral and structural voxel data. Mean ± SD estimates of linewidths, signal-to-noise ratios, CRLB, and voxel proportions of white matter, grey matter and CSF in the hippocampus, anterior cingulate cortex and thalamus in oxytocin and placebo conditions.

Spectral and structural voxel data

	Hippocampus ($N=26$)			Anterior cingulate cortex ($N=28$)			Thalamus ($N=28$)		
	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
FWHM	0.07 ± 0.02	0.07 ± 0.03	$t(25)=-1.12$, $p=.27$	0.04 ± 0.01	0.04 ± 0.01	$t(27)=-0.37$, $p=.71$	0.05 ± 0.02	0.05 ± 0.01	$t(27)=-0.63$, $p=.53$
SNR	15.54 ± 3.40	15.15 ± 2.59	$t(25)=0.60$, $p=.56$	36.07 ± 6.09	34.79 ± 5.72	$t(27)=1.21$, $p=.24$	21.0 ± 3.21	20.64 ± 2.83	$t(27)=0.82$, $p=.42$
WM	0.36 ± 0.07	0.36 ± 0.09	$t(25)=-0.25$, $p=.81$	0.08 ± 0.02	0.09 ± 0.06	$t(27)=-1.15$, $p=.26$	0.78 ± 0.08	0.76 ± 0.08	$t(27)=0.71$, $p=.48$
GM	0.60 ± 0.06	0.60 ± 0.07	$t(25)=0.17$, $p=.87$	0.65 ± 0.05	0.64 ± 0.08	$t(27)=0.85$, $p=.40$	0.22 ± 0.07	0.23 ± 0.08	$t(27)=-0.80$, $p=.43$
CSF	0.04 ± 0.02	0.04 ± 0.02	$t(25)=0.41$, $p=.69$	0.27 ± 0.05	0.27 ± 0.06	$t(27)=0.51$, $p=.62$	0.005 ± 0.007	0.004 ± 0.005	$t(27)=0.58$, $p=.57$

Cramér-Rao Lower Bounds (CRLB)

	Hippocampus ($N=26$)			Anterior cingulate cortex ($N=28$)			Thalamus ($N=28$)		
Metabolite	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
Glu	9.62 ± 2.17	8.92 ± 1.62	$t(25)=1.79$, $p=.09$	5.04 ± 0.43	5.18 ± 0.43	$t(27)=-1.07$, $p=.29$	9.25 ± 2.27	9.21 ± 2.11	$t(27)=0.06$, $p=.96$
Glx	10.65 ± 2.73	10.15 ± 2.22	$t(25)=0.78$, $p=.44$	5.54 ± 0.79	5.82 ± 0.61	$t(27)=-1.49$, $p=.15$	10.61 ± 2.47	11.18 ± 3.54	$t(27)=-0.68$, $p=.50$
NAA	3.62 ± 0.98	3.88 ± 1.61	$t(25)=-0.70$, $p=.49$	2.21 ± 0.42	2.39 ± 0.50	$t(27)=-1.72$, $p=.10$	2.93 ± 0.90	2.93 ± 0.60	$t(27)=0.00$, $p=1.00$
TCho	3.54 ± 0.76	3.65 ± 0.89	$t(25)=-0.68$, $p=.50$	2.71 ± 0.53	2.82 ± 0.61	$t(27)=-0.83$, $p=.42$	3.14 ± 0.45	3.25 ± 0.44	$t(27)=-1.14$, $p=.26$
ml	5.04 ± 0.82	5.46 ± 1.61	$t(25)=-1.49$, $p=.15$	4.14 ± 0.76	4.11 ± 0.88	$t(27)=0.19$, $p=.85$	6.50 ± 1.43	7.29 ± 3.23	$t(27)=-1.25$, $p=.22$
Cre	3.50 ± 0.65	3.73 ± 0.83	$t(25)=-1.36$, $p=.18$	2.07 ± 0.38	2.07 ± 0.26	$t(27)=0.00$, $p=1.00$	3.14 ± 0.45	3.18 ± 0.39	$t(27)=-0.37$, $p=.71$

Abbreviations: FWHM, full width at half-maximum (linewidth) in ppm (parts per million); WM, white matter; GM, grey matter; CSF, cerebrospinal fluid; CRLB, Cramér-Rao Lower Bounds; SNR, signal-to-noise ratio; Glu, Glutamate; Glx, Glutamate + Glutamine; NAA, N-acetylaspartate; TCho, total choline; ml, myo-inositol; Cre, Creatine.

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Table 3 Creatine-scaled and tissue-corrected metabolite values in the hippocampus, anterior cingulate cortex and thalamus (mean ± SD).

Scaled to creatine

Metabolite	Hippocampus ($N=26$)			Anterior cingulate cortex ($N=28$)			Thalamus ($N=28$)		
	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
Glu/Cre	1.01 ± 0.16	1.07 ± 0.17	$t(25)=-1.47$, $p=.15$	1.33 ± 0.11	1.36 ± 0.16	$t(27)=-0.74$, $p=.46$	0.98 ± 0.18	0.98 ± 0.16	$t(27)=-0.01$, $p=1.00$[a]
Glx/Cre	1.40 ± 0.24	1.42 ± 0.24	$t(25)=-0.31$, $p=.76$	1.70 ± 0.17	1.72 ± 0.23	$t(27)=-0.45$, $p=.66$[a]	1.19 ± 0.28	1.24 ± 0.27	$t(27)=-0.54$, $p=.59$
NAA/Cre	1.24 ± 0.19	1.23 ± 0.16	$t(25)=0.03$, $p=.98$[a]	1.24 ± 0.09	1.24 ± 0.08	$t(27)=-0.09$, $p=.93$	1.59 ± 0.15	1.58 ± 0.19	$t(27)=0.25$, $p=.81$
TCho/Cre	0.32 ± 0.03	0.33 ± 0.02	$t(25)=-1.11$, $p=.28$	0.25 ± 0.02	0.24 ± 0.02	**$t(27)=2.88$, $p=.008$***	0.30 ± 0.03	0.29 ± 0.02	$t(27)=1.63$, $p=.12$
mI/Cre	0.90 ± 0.12	0.86 ± 0.11	$t(25)=1.35$, $p=.19$[a]	0.74 ± 0.07	0.75 ± 0.06	$t(27)=-0.82$, $p=.42$[a]	0.58 ± 0.09	0.56 ± 0.11	$t(27)=0.76$, $p=.45$[a]

Corrected for voxel tissue content

Metabolite	Hippocampus ($N=26$)			Anterior cingulate cortex ($N=28$)			Thalamus ($N=28$)		
	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
Glu	7.44 ± 1.33	7.66 ± 1.04	$t(25)=-0.69$, $p=.50$	15.43 ± 1.62	15.43 ± 1.74	$t(27)=0.02$, $p=.99$	7.30 ± 1.39	7.28 ± 1.12	$t(27)=0.05$, $p=.96$[a]
Glx	10.37 ± 2.08	10.19 ± 1.81	$t(25)=0.31$, $p=.76$	19.65 ± 2.51	19.51 ± 2.53	$t(27)=0.27$, $p=.79$[a]	8.88 ± 2.06	9.16 ± 1.96	$t(27)=-0.48$, $p=.64$
NAA	9.05 ± 0.90	8.80 ± 0.84	$t(25)=1.18$, $p=.25$[a]	14.33 ± 1.12	14.15 ± 1.23	$t(27)=0.85$, $p=.40$[a]	11.75 ± 0.87	11.62 ± 0.69	$t(27)=0.76$, $p=.46$
TCho	2.38 ± 0.29	2.37 ± 0.41	$t(25)=0.12$, $p=.90$[a]	2.93 ± 0.36	2.77 ± 0.41	**$t(27)=2.57$, $p=.02$****	2.19 ± 0.22	2.13 ± 0.20	$t(27)=1.38$, $p=.18$[a]
ml	6.68 ± 1.28	6.21 ± 1.12	$t(25)=1.57$, $p=.13$	8.57 ± 1.11	8.57 ± 1.05	$t(27)=-0.01$, $p=.99$	4.29 ± 0.62	4.20 ± 0.93	$t(27)=0.46$, $p=.65$[a]
Cre	7.42 ± 0.86	7.25 ± 1.13	$t(25)=0.73$, $p=.47$[a]	11.60 ± 1.05	11.42 ± 0.96	$t(27)=1.15$, $p=.26$[a]	7.44 ± 0.44	7.44 ± 0.72	$t(27)=-0.05$, $p=.96$

Abbreviations: Glu, Glutamate; Glx, Glutamate + Glutamine; NAA, N-acetylaspartate; TCho, total choline; ml, myo-inositol; /Cre, scaled to Creatine.

[a] Removal of outliers made no material change to the results or conclusions.

* Significant at the Bonferroni-corrected $p<.01$ threshold.

** Although significant at the $p<.05$ level, this does not meet the Bonferroni-corrected $p<.01$ threshold.

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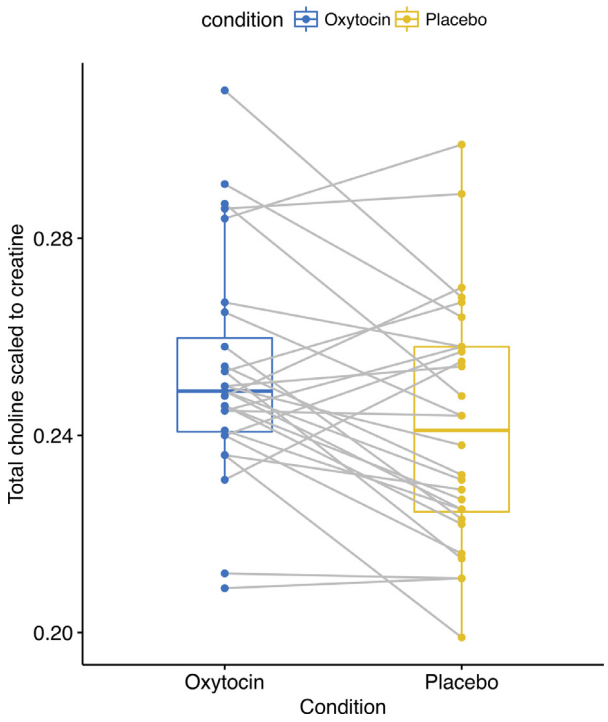

Fig. 3 Choline levels (scaled to creatine) in the anterior cingulate cortex in oxytocin and placebo conditions. Individual data points are presented (overlaid) on standard boxplots, with grey lines connecting paired values.

3.6.2. Attenuated psychotic symptoms

One subject had one item missing out of the four items that constitute the positive subscale of the CAARMS, leaving $N = 25$ for the hippocampus, $N = 27$ for the ACC and $N = 27$ for the thalamus. There were no significant correlations between CAARMS attenuated psychotic symptom scores and change in glutamate or Glx scaled to creatine in the hippocampus, ACC or thalamus (Supplementary Table S2).

4. Discussion

This is the first study to investigate the neurochemical effects of a single dose of oxytocin in CHR-P individuals. The key finding was that oxytocin did not modulate glutamate (or glutamate plus glutamine; Glx) levels in the hippocampus, ACC or thalamus in the time interval examined (approximately 75-99 min post-dosing). There were also no effects on other metabolites, with the exception of choline in the ACC, concentrations of which were significantly increased after oxytocin.

Our primary hypothesis, that an acute dose of oxytocin would modulate glutamate or Glx levels in CHR-P individuals, was not verified. One implication of these findings relates to the potential mechanism of oxytocin's effects. A previous study found that an acute dose of intranasal oxytocin had marked effects on cerebral perfusion (Paloyelis et al., 2016) across regions that show neurochemical alterations in CHR-P groups (Merritt et al., 2016; Poels et al., 2014; Tandon et al., 2013). Our own work has also revealed that acute oxytocin modulates hippocampal perfusion in those at CHR-P (Davies et al., 2019). Given these demonstrable neural effects, in this study we sought to test the hypothesis that oxytocin also modulates metabolite concentrations (especially glutamate and Glx) in CHR-P individuals. Negative findings are underreported in the scientific literature and can be difficult to interpret, but three different possibilities are presented below. First, while we did not find any effect on glutamate or Glx levels in the hippocampus, ACC or thalamus, it is still possible that oxytocin mediates its effects via modulation of glutamatergic neurotransmission either (a) directly, but we are not able to detect it using ^{1}H-MRS (e.g., due to its lack of specificity for neuronal glutamate/Glx and large voxel size), or (b) indirectly via modulation of GABA and associated neural microcircuits, which was not directly quantifiable in the current study. Indeed, some preclinical studies have shown that exogenous oxytocin administration does not alter basal glutamate levels per se, but blocks or attenuates changes in glutamate induced by deleterious manipulations such as drug-induced deficits and restraint stress (Qi et al., 2009, 2012)—while it can directly alter basal GABA levels (Qi et al., 2012). It may also be the case that the heterogeneity inherent in CHR-P samples, both clinically and in relation to regional metabolite concentrations at the specific time of scanning (which may differ between those who will and will not later transition, and those with good vs. poor functional outcomes (Bossong et al., 2018)), may dilute and obscure observable oxytocin effects on glutamate when measured at group level. Stratification of CHR-P samples in future studies would—in theory—allow exploration of this possibility.

A second possible explanation for these negative findings may be that the time interval between intranasal administration of oxytocin and the onset of the ^{1}H-MRS acquisition was too long and potential effects on glutamate/Glx were missed. The mean ± SD of the time since intranasal administration for each region in the current study was: thalamus (75 ± 4 min), ACC (84 ± 5 min), and hippocampus (93 ± 6 min). Early—albeit indirect—evidence suggested that the neural effects of intranasal oxytocin would last at least as long as our data sampling intervals. For example, notwithstanding the extremely small sample, previous work reported that following intranasal administration, the oxytocin signal in cerebrospinal fluid only begins to significantly increase at 75 min post-dosing (Striepens et al., 2013). Another study found that while the effects of oxytocin on human cerebral blood flow peak at 39-51 min post-administration (with a gradual diminution thereafter), they observed sustained perfusion effects over the entire post-treatment interval (up to 78 min) (Paloyelis et al., 2016). However, more recent work suggests that the effects of oxytocin vary as a function of method of administration, brain region, dosage and time since dosing (Martins et al., 2019). For example, in one study of healthy males, oxytocin's effects on amygdala inhibition were observed only between 45-70 min post-administration, and not before or after (Spengler et al., 2017). A forthcoming study (Martins et al., 2019) using a double-blind, placebo-controlled, crossover procedure and comparing various methods of oxytocin administration, indicates that oxytocin-induced decreases in amygdala perfusion are present only within the first ~15-30 min post-dosing. Furthermore, we recently showed that in the same CHR-P sample used in the current

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study, the effects of oxytocin on hippocampal perfusion were more robust in the earlier (22–28 min) vs the later (30–36 min) post-dosing interval (Davies et al., 2019). It may therefore be that in the hippocampus, thalamus and ACC, changes in glutamate and Glx are occurring closer to the time of administration.

Third, the observed lack of effects (especially on glutamate and Glx) may be related to the acute vs. repeated (longer-term) oxytocin dosing regimen. A recent study found that repeated administration of 48IU/day intranasal oxytocin over 6 weeks in people with autism spectrum disorder significantly decreased N-acetylaspartate and Glx levels in the medial prefrontal cortex (Benner et al., 2018), effects that were not observed after 24IU acute challenge (Aoki et al., 2015). Akin to the current work, both of the aforementioned studies were double-blind, placebo-controlled, crossover designs (Aoki et al., 2015; Benner et al., 2018). In addition, their mouse model showed that repeated—but not acute—oxytocin administration significantly downregulated Nr2b (a subunit of the glutamate NMDA receptor) transcript expression, while acute administration reduced levels of molecules associated with the oxytocinergic system (oxytocin mRNA) and with neural activity (immediate early genes; cFos and Arc), but not Nr2b (Benner et al., 2018). In light of these findings, it is possible that longer-term treatment with oxytocin would have had significant effects on the glutamate system, with altered metabolite levels detectable using ^{1}H-MRS.

A final potential contributing factor for our lack of observed effects on glutamate and Glx relates to statistical power. The current study had a relatively large sample size for a neuroimaging-based, within-subject drug challenge study. However, we cannot rule out the possibility that oxytocin had effects of smaller magnitude (e.g., Cohen's D less than ~0.56), which corresponds to a maximum percent change in metabolite (glutamate or Glx) levels of between ~13–20% (depending on the brain region).

Our secondary hypothesis was that oxytocin would modulate metabolites that have been found to be altered in CHR-P and other high-risk groups (Bossong et al., 2018; Stone et al., 2009; Tandon et al., 2013). We found that, at ~75–99 min post-dosing, oxytocin had no effects on concentrations of N-acetylaspartate, myo-inositol or creatine in the hippocampus, ACC or thalamus. The poor availability of previous literature hinders interpretation of these results and as discussed above, we may have had insufficient power to test for effects on these metabolites. We also found that oxytocin had no effects on choline in the hippocampus and thalamus, but did have an effect on choline in the ACC, which was significantly increased after oxytocin. Choline has a two-fold greater concentration in glial cells compared to neurons (Urenjak et al., 1993), is a precursor and metabolite of acetylcholine, and as an essential component of membrane phospholipids is considered a marker of membrane turnover and integrity (Bertolino and Weinberger, 1999). Previous evidence has implicated altered choline in the early phases of psychosis. Increased regional choline levels have been found in patients with first-episode psychosis (Plitman et al., 2016) and schizophrenia (Bustillo et al., 2014) and is associated with a longer duration of untreated illness (Théberge et al., 2004)—potentially due to glutamatergic excitotoxicity causing cell damage (Gasull et al., 2000), resulting in elevated choline from increased cell membrane turnover (Bertolino and Weinberger, 1999; Théberge et al., 2004), or potentially due to increased astrocytic turnover of glutamatergic compounds (Bustillo et al., 2014; Plitman et al., 2016). There is also specific evidence suggesting that abnormal choline levels characterise populations at risk of psychosis. Earlier work showed that choline levels are altered in familial high risk individuals vs. low risk controls in the hippocampus (Capizzano et al., 2011) and in the ACC (Tandon et al., 2013), with ACC increases correlating with attenuated psychotic symptom severity and schizotypy (Tandon et al., 2013). Within CHR-P individuals, ACC choline levels are increased in those who go on to transition vs. those who do not (Jessen et al., 2006)—although CHR-P differences are not always found (Stone et al., 2009; Uhl et al., 2011). There is also evidence demonstrating an effect of oxytocin on choline in the ACC. A previous study conducted in males with autism spectrum disorder found that acute administration of oxytocin modulated levels of choline—but not any other metabolite—in the medial prefrontal cortex/ACC, albeit at a relaxed statistical theshold (Aoki et al., 2015). However, it is important to note that effects on choline were not within our primary hypothesis and these findings should, therefore, be interpreted with caution. Despite similar results in other neurodevelopmental disorders (Aoki et al., 2015), further independent replication studies are needed to validate this finding. The stringent correction for multiple comparisons (implemented because our primary hypothesis was for glutamate and Glx only) may also account for the (statistically) discordant choline results from the creatine-scaled vs. the voxel tissue content-corrected methods.

This study has some limitations. First, ^{1}H-MRS is not able to distinguish between intracellular vs. extracellular (or neuronal vs. non-neuronal) metabolites, and rather represents a whole tissue measure within the specified voxel (Jelen et al., 2018). This leaves the possibility that oxytocin modulated glutamate/Glx specifically in the neuronal component (i.e. related to neurotransmission/pyramidal neurons—which represents a very small proportion of the total glutamate in brain tissue—in the region of uM concentrations) vs. the non-neuronal component (i.e., within glia), which cannot be discriminated. Nevertheless, ^{1}H-MRS at 3 Tesla is widely used to quantify glutamate and Glx and the data acquired in the current study were of high quality. We therefore think it unlikely that acute oxytocin has effects on neurochemical metabolite concentrations other than choline in CHR-P individuals (at least at ~75–99 min post-dosing) and propose that this absence of effects is not attributable to methodological shortcomings. Scanning at higher field strengths (e.g., 7 Tesla) and advanced techniques (such as GluCEST or ^{1}H-fMRS) are now becoming available, and will enable future research to reliably separate spectral components and investigate dynamic changes in metabolite levels (Jelen et al., 2018; Roalf et al., 2017; Thakkar et al., 2017). Our use of metabolite values scaled to creatine could also have meant that the results were caused by changes in voxel creatine levels. We overcame this limitation by also presenting water-scaled metabolite levels corrected for voxel tissue content. Furthermore, while we recruited a representative sample of CHR-P males as typically found in specialist CHR-P services (Fusar-Poli

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et al., 2013b), none of which were taking antipsychotics, a number of participants were taking antidepressants and benzodiazepines. However, we found no material change in the results after exclusion of these cases, aside from an increase in thalamic choline after oxytocin. Future oxytocin studies should also investigate effects in females (which we excluded due to the known sexual dimorphism in oxytocinergic function (Rilling et al., 2014)) and may want to explore variation in the oxytocin receptor gene, which is likely to modulate response to intranasal oxytocin (Tost et al., 2010). Finally, an interesting caveat is that while previous studies in autism spectrum disorder found no absolute differences in metabolite levels after acute oxytocin beyond choline in the ACC, oxytocin's effects on medial prefrontal N-acetylaspartate levels were related to the oxytocin-induced recovery in functional MRI signal in the same region during a social cognition fMRI task, and improvement in social-communication related symptoms (Aoki et al., 2015). Examining whether changes in brain activation or functional connectivity after oxytocin are associated with oxytocin's effects on metabolites in CHR-P patients remains an avenue for future research.

5. Conclusions

This study suggests that at ~75–99 min post-dosing, acute administration of oxytocin does not alter levels of glutamate, glutamate+glutamine, N-acetylaspartate, myo-inositol or choline in the hippocampus, ACC, or thalamus in those at CHR-P, aside from potential effects on choline in the ACC.

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Author contributions

All authors made substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data. Authors PFP, PMcG, DT, SW, PMo, SS, PA, FZ, YP designed the study, wrote the protocol, and/or obtained funding. Authors CD, GR, ADM, VRC, UP, MC, ES, DO, SM, DJL conducted study recruitment, data collection, or provided administrative or clinical support. Author CD conducted the analysis in consultation with PFP and JMS. Author CD wrote the first draft of the manuscript which was revised by PFP. All authors contributed to and have approved the final manuscript.

Conflict of interest

PFP has received advisory consultancy fees from Lundbeck outside of this work. The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.03.008.

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6.2. SUPPLEMENTARY MATERIAL

NEUROCHEMICAL EFFECTS OF OXYTOCIN IN PEOPLE AT CLINICAL HIGH RISK FOR PSYCHOSIS

Cathy Davies, Grazia Rutigliano, Andrea De Micheli, James M Stone, Valentina Ramella-Cravaro, Umberto Provenzani, Marco Cappucciati, Eleanor Scutt, Yannis Paloyelis, Dominic Oliver, Silvia Murguia, Fernando Zelaya, Paul Allen, Sukhi Shergill, Paul Morrison, Steve Williams, David Taylor, David J Lythgoe, Philip McGuire, Paolo Fusar-Poli

– SUPPLEMENTARY MATERIAL –

Supplementary Experimental Procedures

Subjects were asked to abstain from using recreational drugs for at least one week prior to each MRI scan, and alcohol for at least 24 hours prior to each MRI scan. Urine screening was conducted before the scan for each participant.

The blinded spray bottles used for each session (containing oxytocin or matched placebo; see section below for formulation details) were visually identical and dispensed by the Maudsley Hospital Pharmacy. Intranasal administration followed recommended guidelines (Guastella *et al*, 2013) and a protocol adopted by a previous study conducted at our institute (Paloyelis *et al*, 2016). After a demonstration of the intranasal administration from a researcher using a spray bottle containing water, participants self-administered (in the presence of and with feedback from a researcher) one puff (4IU) of intranasal oxytocin or placebo every 30 seconds, alternating between nostrils, until 40IU (10 puffs) had been administered. The administration phase lasted approximately 4.5 minutes.

Both the participants and researchers were blind to the (crossover) treatment sequence (AB or BA) allocation. A randomisation list was generated by the Maudsley Hospital Pharmacy, which determined whether a participant received oxytocin or placebo in their first study visit and vice versa for the second study visit. On recruitment of a study participant, an unblinded clinical trial pharmacist, who was not involved with the rest of the study, allocated the participant to one of the two sequences (AB, BA) based on the randomisation list. Allocation information was kept concealed in the Maudsley Hospital Pharmacy.

Oxytocin and Placebo Formulation

The finished intranasal product was manufactured by the Pharmacy Manufacturing Unit, Guy's and St Thomas' NHS Foundation Trust. Active (Oxytocin): Syntocinon was obtained as Syntocinon Spray, marketed by Hersteller, 68330 Huningue, France. This was decanted into a 10ml amber dropper bottle and capped with a 10IU white nasal atomiser with 69mm dip and overcap. Placebo was placed in the same containers as above and contained excipients matching the active formulation.

Exploratory correlations

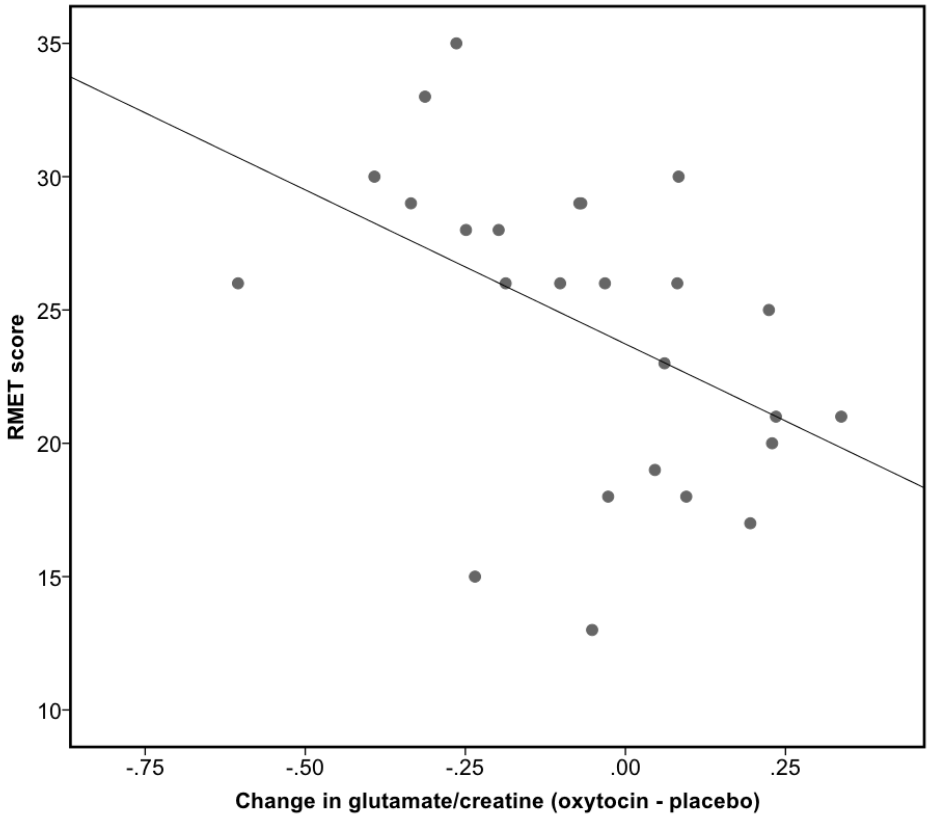
Social Cognition

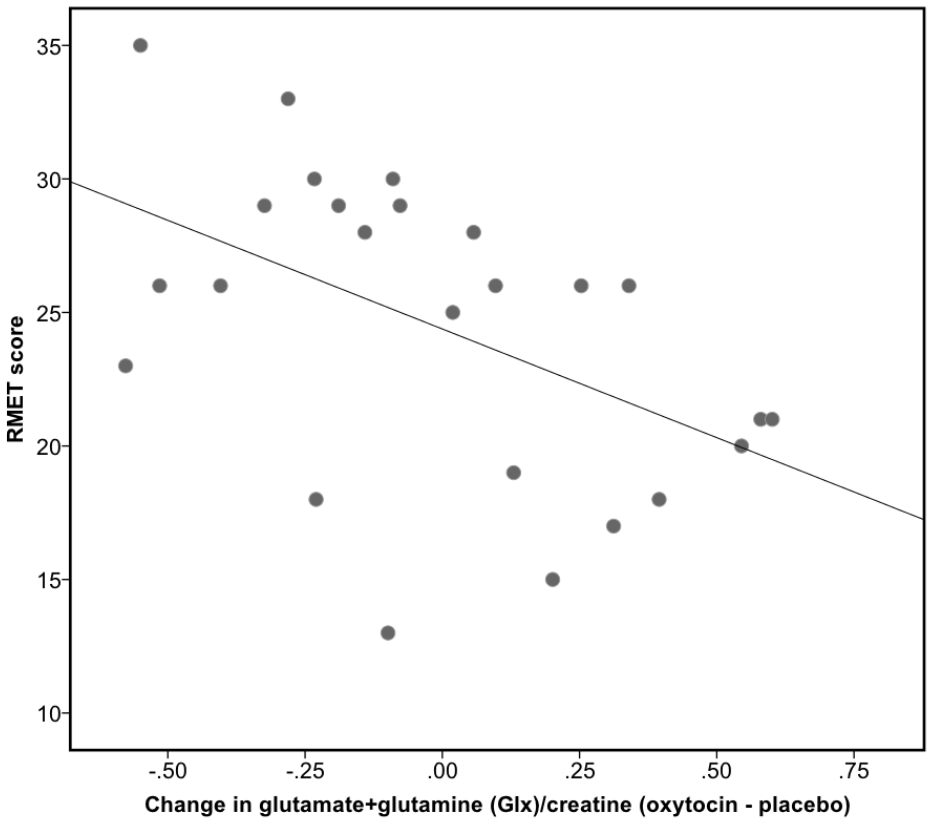
Table S1. Spearman's correlation coefficients for change in metabolites (oxytocin – placebo) in the hippocampus, ACC and thalamus vs RMET scores

Metabolite	Spearman's Rho for RMET	Sig. (2-tailed)	N
Hippocampus			
Change in Glu/Cre	-.546**	.005	25
Change in Glx/Cre	-.509**	.009	25
ACC			
Change in Glu/Cre	.288	.145	27
Change in Glx/Cre	-.035	.861	27
Thalamus			
Change in Glu/Cre	.120	.551	27
Change in Glx/Cre	.232	.245	27

** Correlation is significant at the 0.01 level (2-tailed).

Figure S1. Scatterplots depicting relationship between change in glutamate and Glx scaled to creatine (oxytocin – placebo) in the hippocampus vs RMET scores





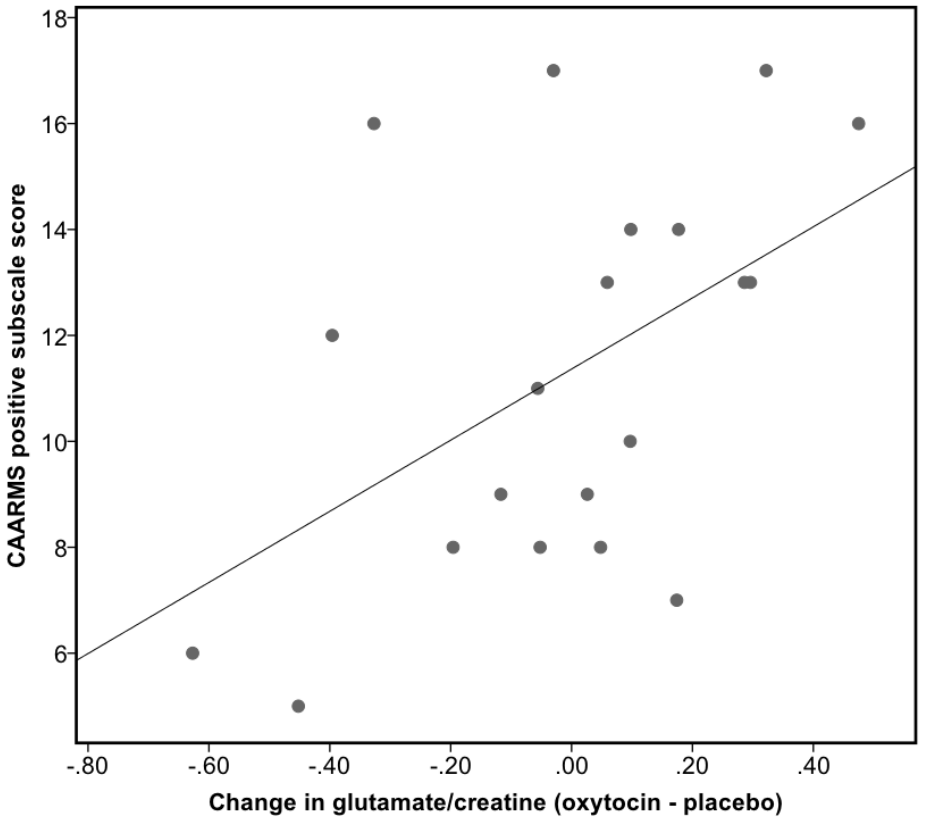
Attenuated Psychotic Symptoms

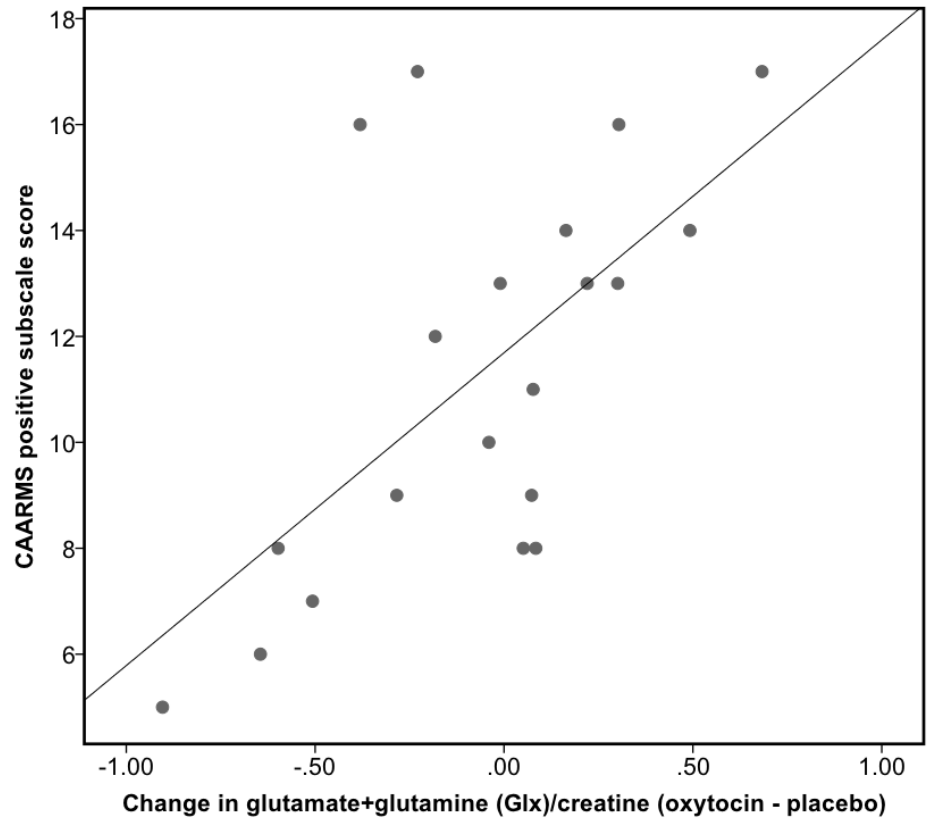
Table S2. Spearman's correlation coefficients for change in metabolites (oxytocin – placebo) in the hippocampus, ACC and thalamus vs CAARMS positive subscale severity scores

Metabolite	Spearman's Rho for CAARMS	Sig. (2-tailed)	N
Hippocampus			
Change in Glu/Cre	-.326	.111	25
Change in Glx/Cre	-.211	.311	25
ACC			
Change in Glu/Cre	.043	.831	27
Change in Glx/Cre	.175	.383	27
Thalamus			
Change in Glu/Cre	.344	.079	27
Change in Glx/Cre	.336	.087	27

There were no significant correlations between CAARMS positive scores and change in glutamate or Glx scaled to creatine in the hippocampus, ACC or thalamus (Table S2). However, after removing those subjects taking antidepressants and benzodiazepines in sensitivity analyses, there was a significant positive association between CAARMS positive scores and change in thalamic glutamate scaled to creatine (rho= .523, p=.018; N=20) and change in thalamic Glx scaled to creatine (rho= .606, p=.005; N=20)(Figure S2). That is, higher levels of positive symptoms were associated with increased glutamate (and Glx) after oxytocin, while lower levels of positive symptoms were associated with a reduction of thalamic glutamate (and Glx) after oxytocin vs placebo (Figure S2).

Figure S2. Scatterplots depicting relationship between change in glutamate and Glx scaled to creatine (oxytocin – placebo) in the thalamus vs CAARMS positive subscale severity scores [after excluding those subjects taking antidepressants or benzodiazepines in sensitivity analyses]





Updated Power Calculations

Updated power calculations were computed using the mean and SD of control metabolite values from a previous study (Stone *et al*, 2009). All calculations are for paired t-tests (two-tailed; 80% power; a=.05), where the assumed SD is the same for both sessions: $SD_{diff} = \sqrt{2} \times SD$. The final sample sizes in the current study were hippocampus=26, ACC=28, thalamus=28.

Overall, the updated power calculations indicated that the minimum effect size (Cohen's D) for within-subject change in glutamate (detectable at 80% power when a=.05), was d=0.57 (~18% change in glutamate levels) in the hippocampus, d=0.55 (~13% change) in the ACC, and d=0.55 (~13% change) in the thalamus. The same values for Glx were: d=0.57 (~20% change in Glx) in the hippocampus, d=0.55 (~14.5% change) in the ACC, and d=0.55 (~19% change) in the thalamus. Therefore, it is possible that oxytocin induced changes of smaller magnitude but we were unable to detect them with our final sample sizes due to low statistical power. Detailed power calculations are presented in Table S3 below.

Table S3. For each Cohen's D effect size (small, medium, large), showing the associated % change in metabolite, the power we had in the current study to detect this % change (or D) [given the sample size for each region], and the *required* sample size to detect such an effect size

Cohen's D	Glutamate			Glx		
	% change in metabolite	Power in **this study** to detect D	Required N	% change in metabolite	Power in **this study** to detect D	Required N
Hippocampus (this study N=26)						
Small (d=0.2)	7	18%	176	7	16%	210
Medium (d=0.5)	16	66%	36	18	69%	34
Large (d=0.8)	26	97%	15	29	98%	15
ACC (this study N=28)						
Small (d=0.2)	5	19%	180	5	16%	223
Medium (d=0.5)	12	73%	33	13	71%	35
Large (d=0.8)	19	98%	15	21	98%	15
Thalamus (this study N=28)						
Small (d=0.2)	5	18%	186	7	18%	195
Medium (d=0.5)	12	72%	34	17	71%	35
Large (d=0.8)	19	98%	15	28	98%	15

From Table S3 it becomes clear that effects of oxytocin would have to be of medium to large magnitude (as defined by Cohen's D) for us to detect them with our final sample sizes.

Supplementary References

Guastella AJ, Hickie IB, McGuinness MM, Otis M, Woods EA, Disinger HM, *et al* (2013). Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research. *Psychoneuroendocrinology* **38**: 612–625.

Paloyelis Y, Doyle OM, Zelaya FO, Maltezos S, Williams SC, Fotopoulou A, *et al* (2016). A Spatiotemporal Profile of In Vivo Cerebral Blood Flow Changes Following Intranasal Oxytocin in Humans. *Biol Psychiatry* **79**: 693–705.

Stone JM, Day F, Tsagaraki H, Valli I, McLean M a., Lythgoe DJ, *et al* (2009). Glutamate Dysfunction in People with Prodromal Symptoms of Psychosis: Relationship to Gray Matter Volume. *Biol Psychiatry* **66**: 533–539.

PART 3 – GENERAL DISCUSSION

7. DISCUSSION

7.1. SUMMARY OF FINDINGS

Using two distinct but complementary research approaches, the studies within this thesis set out to advance our understanding of current (and previously tested) interventions [Part 1] and a potentially novel treatment strategy [Part 2] for patients at CHR-P. The objective of **Part 1** was to apply more sophisticated (network) meta-analytical methods than have been previously used to synthesise and summarise the evidence regarding the efficacy and acceptability of treatments for (a) preventing transition to psychosis from a CHR-P state, and (b) reducing attenuated positive psychotic symptoms. The objective of **Part 2** was to use a placebo-controlled pharmacological challenge and MRI to examine whether a potential novel treatment strategy, the neuropeptide oxytocin, engaged two pathophysiological targets linked to psychosis onset, primarily (a) hippocampal perfusion, and (b) glutamate (and Glx) concentrations in the hippocampus, ACC and thalamus. Without repeating the discussions of the individual papers, in the sections that follow I will discuss these findings in context with the wider literature, consider the implications for research and clinical practice, review methodological strengths and limitations and suggest areas for future investigation.

The key finding from **Part 1** is that at present, there is no evidence that any specific treatment is superior to any other treatment—including the lowest level needs-based intervention—in preventing transition to psychosis (**Paper 1**), reducing attenuated positive psychotic symptoms (**Paper 2**) or in acceptability. These findings have contributed to knowledge because they were the first to challenge the prevailing view that CBT is somehow superior to all other treatments and should be offered to CHR-P patients as an evidence-based intervention (NICE, 2014). As will be discussed in the following sections, the studies within Part 1 have also allowed identification of numerous potential sources of bias and issues with how current clinical trials are conducted, while also proposing ways of overcoming them in the next generation of research.

The main findings from **Part 2** are that oxytocin modulates resting hippocampal perfusion in CHR-P patients (**Paper 3**) but does not appear to alter concentrations of glutamate (or Glx) in the hippocampus, ACC or thalamus when measured using 1H-MRS (**Paper 4**). More specifically, in the study of cerebral blood flow, oxytocin was found to increase perfusion in the left hippocampus in a region-of-interest analyses of data collected at 22–28 (run 1) and at 30–36 (run 2) mins post-administration. The effect in run 1 (but not run

2) remained significant after controlling for effects on global blood flow. In exploratory analyses, we found that oxytocin increased perfusion in each of the tested hippocampal subregions: CA1, CA2, CA3, dentate gyrus and subiculum. In addition to the hippocampus, in a whole-brain analysis we also discovered that oxytocin modulated perfusion in further brain regions associated with social and emotional processing (as well as CHR-P pathophysiology), such as the thalamus, fusiform gyrus, parietal cortex and cerebellum. As will be discussed below (section 7.5.1), these findings advance knowledge by demonstrating that acutely administered oxytocin can engage one of the key pathophysiological targets associated with the onset of psychosis in patients at CHR-P, and therefore merits further investigation as a candidate novel treatment.

Conversely, results from the 1H-MRS study (Paper 4) suggested that oxytocin does not modulate the concentrations of glutamate or Glx in the left hippocampus, ACC or left thalamus in CHR-P patients, at least not in the time intervals sampled in the current study (starting 75 to 93 mins post-dosing). The only significant finding from analyses of the remaining metabolites (NAA, choline and myo-inositol) was of increased choline in the ACC after oxytocin. As will be discussed in section 7.5.1 below, these—mostly negative—findings may help us understand the best way of evaluating the effects of oxytocin in these patients in future trials.

7.2. EVIDENCE SYNTHESIS – FINDINGS IN CONTEXT & IMPLICATIONS

7.2.1. THE HYPE CYCLE – THE JOURNEY SO FAR

One way of conceptualising and situating our findings within the historical development and progression of the CHR-P field (and its search for preventive treatments) is using the "Gartner Hype Cycle" (Gartner, 2018) model (adapted to the CHR-P paradigm in (Fusar-Poli, 2018)). The Gartner Hype Cycle describes the course of new technological discoveries, assuming that humans tend to overestimate the impact of a new discovery in the short term, while largely underestimating the same in the long term. The Hype Cycle—illustrated in **Figure 7–1** on page 147—includes 5 different stages: (1) innovation trigger, (2) peak of inflated expectations, (3) trough of disillusionment, (4) slope of enlightenment, and (5) plateau of productivity or knowledge.

Innovation trigger – the beginning

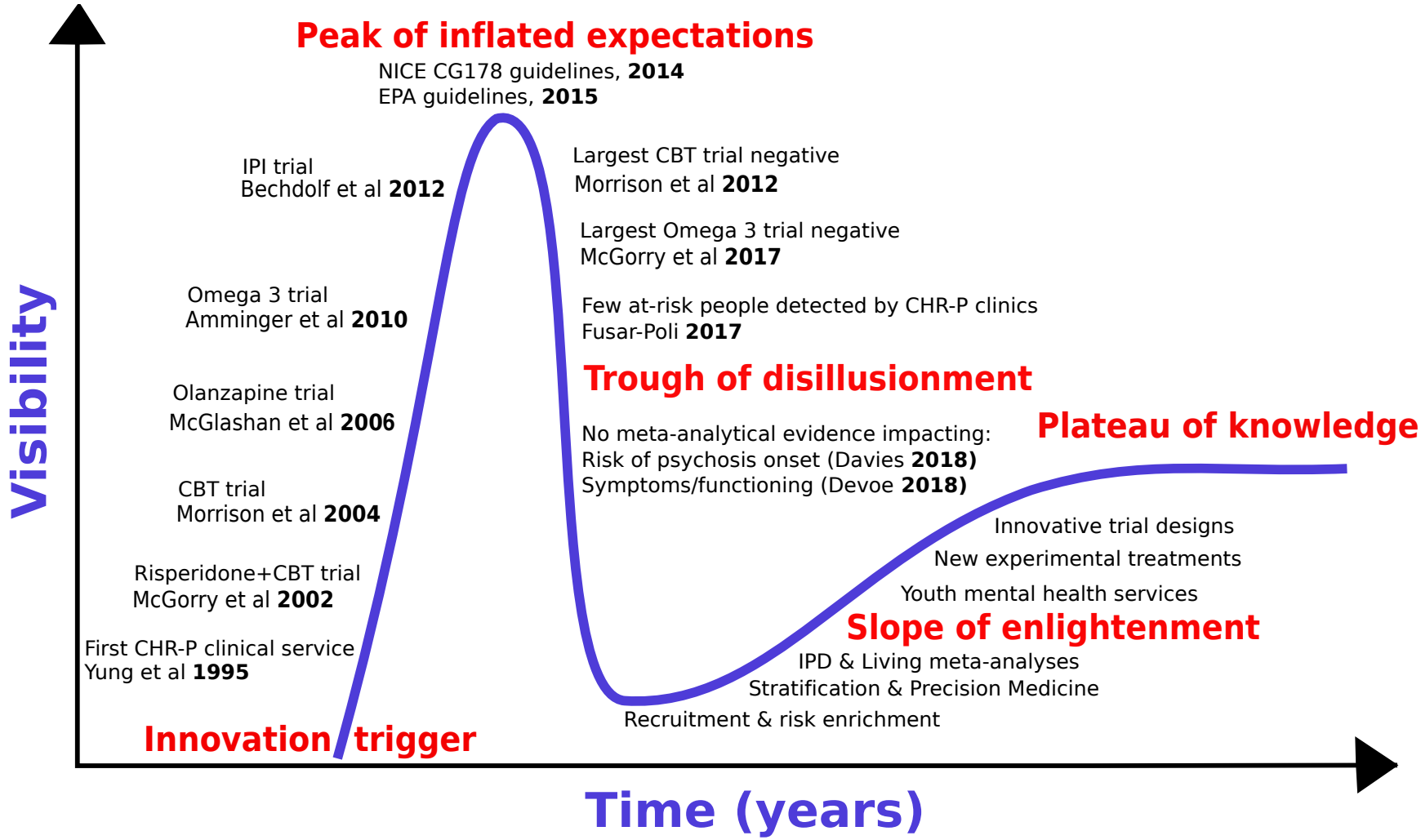
With reference to the CHR-P paradigm, the innovation trigger for the development of preventive treatments for psychosis was the set-up of the first CHR-P clinic in 1995, followed in 2002 by the publication of the first randomised controlled trial in CHR-P

individuals. This trial employed antipsychotic (risperidone) plus psychological treatment (CBT) and demonstrated that they were effective in reducing the risk of developing first-episode psychosis in CHR-P samples (McGorry *et al*, 2002). Over the ensuing years, the innovation trigger led to an explosion of enthusiasm with the development of additional treatments that—it was hoped—could prevent the onset of psychosis from a CHR-P state. As reviewed in full in Part 1 Introduction (section 1.2 starting on page 18), randomised controlled trials involving antipsychotics (olanzapine (McGlashan *et al*, 2006)), psychological therapies (CBT (Morrison *et al*, 2004) and Integrated Psychological Interventions (Bechdolf *et al*, 2012)) and dietary interventions (omega-3 (Amminger *et al*, 2010)) all confirmed some degree of efficacy for reducing the onset of psychosis in CHR-P individuals.

Inflated expectations

The peak of inflated expectations and optimism was likely reached through the publication of the NICE Clinical Guideline 178 (NICE, 2014) and the European Psychiatric Association (EPA) guidelines (Schmidt *et al*, 2015). These guidelines set the gold standard for the prevention of psychosis in CHR-P individuals in clinical routine. At the same time, these guidelines also represented the starting point of the trough of disillusionment, because they were grounded on "non-conclusive" and "moderate quality" evidence of small magnitude (risk difference -0.07, 95% CIs -0.14 to -0.01) (Stafford *et al*, 2013a, 2013b). Upon closer inspection, the two guidelines were already reflecting a lack of robust evidence at the time of their release because they were partially discordant—while prophylactic treatment with antipsychotics was altogether prohibited by NICE guidelines (NICE, 2014), the EPA allowed their use in the case of severe and progressive symptomatology (Schmidt *et al*, 2015).

Figure 7–1. The Gartner hype cycle of preventive treatments for psychosis.



Trough of disillusionment – where we are now

Over the following years, the trough of disillusionment for effective preventative treatments received additional corroborating support. Two large randomised controlled trials showed that neither CBT (Morrison *et al*, 2012) nor omega-3 interventions (McGorry *et al*, 2017; Nelson *et al*, 2018a) were effective in reducing the progression to psychosis from a CHR-P state. Overall, seven new RCTs involving 992 new CHR-P participants (an increase of more than 50%) have been published since the last meta-analysis (Stafford *et al*, 2013a) that informed the NICE guidelines (Davies *et al*, 2018a). All of these new trials were negative (Davies *et al*, 2018a; Fusar-Poli, 2017a). The updated network meta-analysis (Paper 1) incorporating these new RCTs (and using the most sophisticated meta-analytic methods to date) confirmed the lack of evidence to support specific treatments over each—and every—other for the prevention of psychosis in CHR-P individuals (Davies *et al*, 2018a). Paper 2 of this thesis (Davies *et al*, 2018b), and an independent study using the same methods and outcomes (Devoe *et al*, 2019) also confirmed no superiority of any treatment for reducing attenuated psychotic symptoms.

Our finding that no specific interventions were superior to any others—including the lowest-level 'nonspecific' needs-based intervention—may have been the first evidence synthesis to present such sobering conclusions, but when evaluating the wider literature, these results are perhaps not entirely surprising. This includes the fact that the seven most recent (Bechdolf *et al*, 2016; Cadenhead *et al*, 2017; Kantrowitz *et al*, 2015; McGorry *et al*, 2017; Miklowitz *et al*, 2014; Stain *et al*, 2016; Woods *et al*, 2017) and three largest (Bechdolf *et al*, 2016; McGorry *et al*, 2017; Morrison *et al*, 2012) RCTs all produced non-significant results for preventing transition, and independent meta-analyses conducted outside of our research team have since published concordant findings for additional outcomes (Devoe *et al*, 2018c, 2018a, 2018b, 2019).

7.2.2. COMPARISON WITH RECENT META-ANALYSES – VARIOUS OUTCOMES

To further demonstrate that the findings of Paper 1 and Paper 2 are concordant with the wider literature and across various clinical outcomes, a systematic search of meta-analyses (adapted from Paper 5 of this thesis, which is under review as noted in the Preface) was conducted and details of the most recent meta-analyses were extracted and reported in **Table 7–1** on page 150.

The most updated meta-analytical evidence, summarised in **Table 7–1**, shows no evidence to favour any preventive treatments over each (and any) other for improving any of the following clinical outcomes relevant for CHR-P individuals: risk of developing a first episode of psychosis (Paper 1) (Davies *et al*, 2018a), acceptability of treatments (Paper 1) (Davies *et al*, 2018a), severity of attenuated positive (Paper 2) (Davies *et al*, 2018b; Devoe *et al*, 2019) or negative psychotic symptoms (Devoe *et al*, 2018c, 2018b), depression (Stafford *et al*, 2013a), symptom-related distress (Hutton and Taylor, 2014), level of social functioning (Devoe *et al*, 2018a), level of general functioning (Hutton and Taylor, 2014) or quality of life (Hutton and Taylor, 2014).

Although CHR-P patients may have symptomatic improvement pre-post treatment (and longitudinally in naturalistic settings (Fusar-Poli *et al*, 2015b)), there are no substantial differences across interventions or between intervention and control conditions. Importantly, although the meta-analyses presented in **Table 7–1** were conducted by independent research teams, their results converge. For example, two independent meta-analyses (including Paper 2) both concluded that there is no evidence to favour specific treatments over each (and every) other for improving attenuated psychotic symptoms in CHR-P individuals (Davies *et al*, 2018b; Devoe *et al*, 2019). Unfortunately, there is also no robust evidence that current treatments can improve functioning in these patients. The most recent meta-analysis to have explored this outcome concluded that CBT did not significantly improve social functioning at 6 months, 12 months or 18 months; omega-3 did not significantly improve social functioning at 6 months or 12 months and cognitive remediation did not significantly improve social functioning at 2–3 month follow up (Devoe *et al*, 2018a).

Table 7–1. Efficacy of treatments for CHR-P individuals. Overview of the most recent meta-analyses per outcome (up to January 2019).

Outcome	Author	Year	Type of evidence	N of studies (max N of CHR-P individuals[a])	Finding
Transition to psychosis	Davies et al (Paper 1)	2018	Aggregate Network Meta-Analysis (RCTs)	16 (2035)	Lack of evidence to favour specific treatments
Acceptability	Davies et al (Paper 1)	2018	Aggregate Network Meta-Analysis (RCTs)	14 (1848)	Lack of evidence to favour specific treatments
Severity of attenuated positive symptoms	Davies et al (Paper 2)	2018	Aggregate Network Meta-Analysis (RCTs)	14 (1707)	Lack of evidence to favour specific treatments
	Devoe et al	2018	Aggregate Network Meta-Analysis (RCTs)	12 (1457)[b]	Lack of evidence to favour specific treatments
Severity of attenuated negative symptoms	Devoe et al	2018	Aggregate Network Meta-Analysis (RCTs)	14 (1467)[c]	Lack of evidence to favour specific treatments
Depression	Stafford et al	2013	Aggregate Pairwise Meta-Analysis (RCTs)	5 (714)	No significant treatment effects at any time point
Symptom-related distress	Hutton et al	2014	Aggregate Pairwise Meta-Analysis (RCTs)	Unclear	No significant treatment effects
Social functioning	Devoe et al	2018	Aggregate Pairwise Meta-Analysis (RCTs)	9 (1040)	No treatment significantly improved social functioning
Functioning	Hutton et al	2014	Aggregate Pairwise Meta-Analysis (RCTs)	6 (800)	No significant treatment effects
Quality of life	Hutton et al	2014	Aggregate Pairwise Meta-Analysis (RCTs)	Unclear	No significant treatment effects

[a] sample sizes based on the total sample sizes reported in the meta-analysis minus the sample size of any studies that were not included in their meta-analytic computations; [b] sample size of Ising et al (2016) and the non-randomised arm of McGorry et al 2013 (N=78) not included; [c] sample size computed by summing study sample sizes from Table 1 in the paper. The non-randomised arm of McGorry et al 2013 (N=78) was not included.

7.2.3. THE HYPE CYCLE – THE CHALLENGES TO OVERCOME

Slope of enlightenment

Publication of the negative (non-significant findings) in Papers 1 and 2, along with the accumulating number of failed clinical trials, has prompted critique and discussion about the CHR-P field (van Os and Guloksuz, 2017) as a whole, including whether transition is even a primary outcome of interest and whether treatments are effective for other outcomes. As I will argue in the following sections, rather than taking these findings as evidence to abandon the search for preventative treatments and the CHR-P field, 'negative' findings (such as Papers 1 and 2), and a systematic identification of the limitations of current knowledge, may actually help us to advance the field. This is precisely the approach taken by other medical specialties; the uncertain stage of knowledge typically associated with the "trough of disillusionment" is not specific to the CHR-P state; rather, it has also been observed in other branches of medicine, such as cancer prevention (Cuzick, 2017).

As I will discuss below, implementing the "slope of enlightenment" will also likely require the coordinated efforts of researchers to improve trial designs and pay increased attention to factors such as: (a) ensuring sufficient risk enrichment in study samples, (b) estimation of specific treatment effect estimates rather than clinically uninterpretable pooled effect sizes, and (c) consensus on what constitutes the primary outcome(s) for the field.

7.2.4. THE NEED FOR SPECIFIC TREATMENT EFFECT ESTIMATES

Why were treatments not 'pooled' altogether?

Since preventative treatments for CHR-P individuals are highly heterogeneous, pooled effect sizes (of all different treatments combined) are clinically meaningless and cannot be used to inform treatment guidelines. How would a clinician, who needs to decide on the best treatment to offer to a CHR-P patient, fare when interpreting a pooled effect size which has been estimated across pharmacological, psychological and dietary interventions combined vs heterogeneous control conditions? Such an effect size would, at best, suggest that "any type of experimental treatment" is better, worse, or similar to "any type of control condition". This is clearly uninformative from a clinical standpoint. Treatment guidelines—and therefore the underlying evidence—needs to be as specific as possible because clinicians, healthcare providers and patients rely on them for clear direction and education on the best ways of adhering to them.

Accordingly, the rationale for conducting the first randomised controlled trial in the CHR-P population was based on the need to examine more "specific interventions" (page 922 in (McGorry *et al*, 2002)). It therefore follows that in meta-analyses of CHR-P treatments, it is the examination of specific—as opposed to general—treatment efficacy that is important. Independent research teams have now used network meta-analytical approaches to investigate the specific efficacy of interventions for CHR-P individuals and, importantly, none of them have produced pooled effect sizes across treatment modalities (Davies *et al*, 2018a, 2018b, Devoe *et al*, 2018c, 2019).

Treatment heterogeneity

Interventions for CHR-P patients are intrinsically heterogeneous because they include different therapeutic components. This is due to the fact that when interventions were originally introduced in the first RCT, the strategy adopted was to "include the best-bet specific therapies in a single enhanced intervention package to determine whether it was possible to delay the onset of psychosis" (McGorry *et al*, 2002). The subsequent trials followed this approach by testing different packages of care, each of which was characterised by specific—but differing—therapeutic components. This issue is evident, for example, in the case of different types (and differing protocols) of psychological therapies, which have been defined as "black boxes" (Hartmann *et al*, 2017). The additional and substantial problem with using pooled effect sizes in the CHR-P field is that the control group, traditionally termed "treatment as usual" or "needs-based intervention" (van der Gaag *et al*, 2012), is per se poorly standardised and largely dependent on local service configurations and the availability of specific resources or competences (Davies *et al*, 2018a). For example, treatment as usual may encompass supportive psychotherapy (primarily focusing on pertinent issues such as social relationships and vocational or family problems), case management, providing psychosocial assistance with accommodation, education or employment, brief family psychoeducation and support, medications other than antipsychotics or clinical monitoring and crisis management (McGorry *et al*, 2002; Yung *et al*, 2007).

One way to overcome these issues is to employ network meta-analyses—as in Papers 1 and 2—which are considered the highest level of evidence as recommended by the World Health Organisation (Kanters *et al*, 2016; Leucht *et al*, 2016).

7.2.5. PREVENTING TRANSITION – THE PRIMARY OUTCOME?

Until now, there has been a converging consensus in the CHR-P field that prevention of psychosis was the foremost outcome and the ultimate aim of the entire paradigm. Authors of RCTs in CHR-P individuals have declared that the goals of early detection are: (1) postponement or prevention of the transition to frank psychosis, (2) reduction of the duration of untreated psychosis to a minimum in patients who develop florid psychosis, and (3) prevention of delayed access to mental health services (van der Gaag *et al*, 2012). The importance of preventing the onset of psychosis in CHR-P individuals has been further endorsed worldwide and supported by consensus papers (Fusar-Poli *et al*, 2013a). Notably, the first RCT in these patients aimed at determining whether it was possible to delay the onset of psychosis (McGorry *et al*, 2002), with the rationale that this would be the most important way of altering the course of the disorder and thereby improving the lives of many patients. Outcomes other than the onset of psychosis have also been poorly operationalised in the CHR-P field. For example, there is no clear definition for symptomatic remission or good outcomes in these patients. Such accumulating evidence clearly indicates that the prevention of psychosis has been "the" first-order issue in the CHR-P field.

It also does not seem justified to downgrade the relevance of preventing psychosis in CHR-P individuals just because recent meta-analyses (including Papers 1 and 2) have not found robust evidence to favour specific preventive interventions. Evidence synthesis is conducted to test the robustness of findings on a determinate topic and the reporting of negative findings is equally as important as positive findings. By systematically identifying and addressing gaps in knowledge, "negative" evidence syntheses (such as in Papers 1 and 2) may, in fact, help to advance the field. For example, on closer inspection, the results of Paper 1 concluded that owing to wide confidence intervals, the actual efficacy of treatments for preventing psychosis is mostly undetermined (Davies *et al*, 2018a). Some signal of treatment efficacy may have been missed because of the large clinical heterogeneity of the population being investigated (Radua *et al*, 2018). On a more conceptual level, there is some consensus that psychosis onset as defined categorically is an arbitrary concept (Fusar-Poli *et al*, 2016a, 2017a; Fusar-Poli and Van Os, 2013; McGorry *et al*, 2018) and that it should be refined or complemented by other relevant outcomes, such as severity of attenuated psychotic symptoms, disability and functioning. However, the prevention of psychosis should remain the cornerstone and the most important outcome for the CHR-P field, complemented by other outcomes.

Downgrading—or at worst, dropping—such a primary outcome would be an indirect demonstration that current CHR-P research has approached the lowest depths of the trough of disillusionment, with complete failure in its ability to prevent psychosis. It would also prove that preventive psychiatry is different from other branches of medicine. For example, lack of effective treatments to prevent dementia (van Charante *et al*, 2016) from a mild cognitive impairment stage has not triggered neurologists to claim that prevention of dementia should be downgraded to a second-order issue (rather, it has become a public health priority (Frankish and Horton, 2017)).

7.2.6. UNDERPOWERED TRIALS – THE ESSENTIAL ROLE OF RISK ENRICHMENT

Another factor that will need to be addressed on the slope of enlightenment relates to risk enrichment, i.e., the actual level of risk for psychosis in the study samples being recruited. The psychometric instruments traditionally used to ascertain the presence of a CHR-P state have been validated worldwide and, overall, have demonstrated excellent prognostic accuracy (area under the curve [AUC] at 3 years: 0.9), which is comparable to that of other prognostic instruments employed in medicine (Fusar-Poli *et al*, 2015a). However, this excellent prognostic accuracy is mostly due to the excellent sensitivity (96%) of CHR-P instruments to detect a state of risk for psychosis; they are able to detect nearly all individuals who will develop psychosis, and consequently, nearly all individuals testing negative should be individuals who will not develop psychosis. In other words, a negative CHR-P assessment is associated with a very small probability of developing psychosis (1.56% at 3 years, negative likelihood ratio of 0.09) (Fusar-Poli *et al*, 2015a; Fusar-Poli and Schultze-Lutter, 2016). Conversely, CHR-P instruments have a poor specificity (47%)—among individuals testing positive, most will not develop psychosis, or in other words, a positive CHR-P assessment is still associated with a relatively small probability of developing psychosis (26% at 3 years, positive likelihood ratio of 1.82) (Fusar-Poli *et al*, 2015a; Fusar-Poli and Schultze-Lutter, 2016). Therefore, there is only a limited predictive gain in testing positive on a CHR-P assessment. As a result, CHR-P instruments should be used in samples that have already been enriched in their initial risk for psychosis (i.e. referrals based on suspicion of psychosis risk) and not as screening methods in the general population (see below) (Fusar-Poli, 2017b; Fusar-Poli *et al*, 2015a). Otherwise, the global number of false positives would be so high that most individuals testing positive would be false positives (Fusar-Poli, 2017b).

When individuals undergoing a CHR-P assessment are recruited from mental health services, they have already accumulated several risk factors for the disorder (Fusar-Poli *et al*, 2017b) which increase their level of risk to 15% at 3 years, compared to the 0.43% 3-year risk in the local age-matched general population (Fusar-Poli *et al*, 2018a). This level of risk is also termed "pretest risk" because it is ascertained in the whole group of people undergoing a CHR-P assessment before the results of the assessment itself are known. The pretest risk in individuals recruited through mental health services (i.e. measured in naturalistic studies, excluding randomised controlled trials) is 15% at 3 years, worldwide (Fusar-Poli *et al*, 2016e). When these individuals are assessed, those meeting CHR-P criteria will have a 26% (post-test) risk of developing psychosis at 3 years (1.7-fold increase from the pretest of 15%) and those not meeting CHR-P criteria will have a 1.56% (post-test) risk of developing psychosis at 3 years (10-fold decrease from the pretest risk of 15%) (Fusar-Poli *et al*, 2015a). However, such an estimate is highly variable, with 95% CIs for the pretest risk ranging from 9% to 24% because it is based on unstandardised and idiosyncratic recruitment strategies (Fusar-Poli *et al*, 2016e).

As indicated in **Table 7–2** (on page 157), assuming an alpha of 0.05, power of 80% and two-tailed test, it is possible to estimate the sample size required to test a new experimental treatment for preventing psychosis against treatment as usual (e.g. needs-based intervention). If the experimental treatment is able to halve the risk of psychosis (risk ratio, RR=0.5), 538 CHR-P individuals (conservatively using the 9% lower bound of the 95% CIs to avoid additional underpowered trials (Fusar-Poli, 2017a)) are required to complete the trial. After considering some attrition due to loss to follow-up (e.g. 20%), the final sample size required would be approximately 646 CHR-P individuals. However, this sample size is based on the pretest risk estimate from naturalistic studies that represent the whole population seeking help at specialised CHR-P clinics. When CHR-P individuals detected by these specialised clinics are recruited into randomised controlled trials, it is likely that additional sampling biases would apply, further reducing the risk enrichment. For example, the push towards recruiting sufficient numbers of trial participants within a fixed period of time may lead to more intensive outreach campaigns in the local community, which are well known to dilute the pretest risk of the resulting sample (Fusar-Poli *et al*, 2016d). In other words, when using unstructured recruitment strategies in new trials, 646 CHR-P patients may not provide sufficient power to test preventive effects of magnitude RR= 0.5. If the efficacy of the preventative treatment is lower, for example, if the experimental treatment is able to reduce the risk of psychosis

onset by only 40% (RR=0.6) or 30% (RR=0.7), 894 and 1,680 CHR-P patients are needed (ignoring attrition), respectively.

If individuals undergoing a CHR-P assessment are mostly recruited from the community, they will have accumulated fewer (or no) risk factors for psychosis and their pretest risk would be 0.43% at 3 years. Following the estimates reported in **Table 7–2**, the sample size required for a similar RCT would exceed 13,000 CHR-P individuals (ignoring attrition) and again, CHR-P instruments themselves do not work well when they are applied outside of clinical samples that have already undergone some pretest risk enrichment (Fusar-Poli, 2017b). Thus, as noted above, it is clear that the way individuals are recruited (for undergoing a CHR-P assessment) drives the level of risk enrichment (Fusar-Poli *et al*, 2016e) and ultimately, impacts the statistical power of the trial. For example, the NEURAPRO trial observed a risk of psychosis onset of approximately 14% at 3.4 years in CHR-P individuals who received the control condition, which suggests a pretest risk of 8.2% (assuming that the control condition had little effect on the risk for psychosis) (McGorry *et al*, 2017; Nelson *et al*, 2018a). Under those circumstances, 596 CHR-P individuals were needed to detect a 50% decrease in risk in the experimental condition, which exceeds the sample size employed by the trial. In the case of some effect of the control condition on the risk of transitioning to psychosis (as speculated by the authors (McGorry *et al*, 2017)), the estimated sample size would then be lower.

Table 7–2. Risk enrichment impacts statistical power and sample size for experimental therapeutic trials in CHR-P samples.

Sampling	**Recruitment (pretest)**	**Psychometric assessment (post-test)**		**Total sample size (ignoring attrition)** [(c)]		
Type of sample	**Risk of psychosis at 3 yrs (%)**	**Risk of psychosis at 3 yrs (%)**		**Risk Ratio (risk experimental treatment / risk needs-based intervention)**		
		CHR+ [(a)]	**CHR-** [(b)]	**0.5**	**0.6**	**0.7**
General population	0.43	0.7	<0.1	13,378	22,292	42,100
NEURAPRO control arm	8.2	14	0.8	596	988	1,860
Pretest risk stratification	8	13.7	0.8	610	1,014	1,906
Average pretest risk in people undergoing CHR-P assessment outside RCTs	15 [(d)] (95% CI 9–24)	15.3	0.9	538	894	1,680

(a) LR+ = 1.82; **(b)** LR- = 0.09; **(c)** alpha=0.05; power 80%; 2-sided; allocation ratio = 1. Post-test probability (in %) ={LR*pretest probability/[(1-pretest probability)+(pretest probability*LR)]}*100. The sample sizes reported in the table indicate the individuals who should complete the trial (to estimate the baseline sample, attrition should be considered); **(d)** the average is 15% but because it depends on unstandardised idiosyncratic recruitment strategies (Fusar-Poli *et al*, 2016e), it is highly variable. It tends to be on the lower side when the recruitment focuses on children and adolescent populations, intermediate when the recruitment focuses on primary care settings, and higher when the recruitment focuses on secondary care (Fusar-Poli *et al*, 2016c). To allow a conservative estimate of the sample size required, in this table only the lower bound of 9% is used.

It is therefore possible that the low level of risk for psychosis decreased the statistical power of the NEURAPRO trial for detecting small signal effects associated with the experimental treatment. Lack of statistical power due to poor level of psychosis risk may actually be one of the causes of the negative RCTs in this population (Fusar-Poli, 2017a) and of the associated wide 95% confidence intervals observed in the latest network meta-analyses (including Papers 1 and 2) (Davies *et al*, 2018a, 2018b).

The main problem is that recruitment strategies in this field are idiosyncratic and poorly standardised and as such, it is not possible to control the level of pretest risk enrichment. This is particularly concerning in the case of recruitment into trials which, as noted above, introduces additional selection biases that may further dilute the risk enrichment. For example, likely because of more intense outreach campaigns in the community (e.g. from increasing pressure to recruit participants among many simultaneous competing studies), the actual risk of psychosis in CHR-P samples has been declining from 29% in 2012 (Fusar-Poli *et al*, 2012a) to 20% in 2016 (Fusar-Poli *et al*, 2016b) worldwide. Interestingly, there are exceptions to this phenomenon, such as the Outreach and Support in South London (OASIS) CHR-P service (Fusar-Poli *et al*, 2013b), where transition risk has not declined over time (Fusar-Poli *et al*, 2018c). This is again due to the fact that recruitment strategies have, overall, maintained a stable pretest risk enrichment. Ultimately, innovative strategies are needed to ensure that a sufficient level of risk enrichment is obtained to allow adequate statistical power in future trials.

Risk enrichment - solutions for future research

A possible solution for future research could be to apply risk stratification algorithms that have been developed and validated for this population. For example, pretest risk estimation algorithms based on the source of referral to CHR-P clinics and ethnicity can be used to stratify individuals into four classes of risk enrichment: low risk (1% risk at 2 years, ≈22% of the CHR-P population), moderately-low risk (8% at 2 years, ≈53% of the CHR-P population), moderately-high (18% at 2 years, ≈21% of the CHR-P population), and high (35% at 2 years, ≈4% of the CHR-P population) (Fusar-Poli *et al*, 2016c). If this simple tool is applied to individuals recruited for a CHR-P assessment, and those at low risk are screened out from trial eligibility, a pretest risk enrichment of at least 8% would be ensured. Under those circumstances, a total sample of 610 CHR-P individuals (ignoring attrition) would guarantee sufficient statistical

power (80%) to test treatment effects that can halve the risk of developing psychosis (**Table 7–2** on page 157).

Importantly, however, these estimates still relate to relatively large effect sizes (RR=0.5). Interventional CHR-P studies to date have not been powered to detect smaller effects, and it therefore remains possible that treatments have a preventative effect but of smaller magnitude. This problem arises because the outcome (transition) is infrequent and therefore conducting research (in the hope of finding significant preventative effects) is complex. Nevertheless, controlling pretest risk enrichment through recruitment is a promising method that would help mitigate the existing challenges and facilitate the slope of enlightenment phase (**Figure 7–1** on page 147).

7.2.7. CLINICAL HETEROGENEITY – STRATIFICATION AND PRECISION MEDICINE

As noted above, the lack of evidence to favour any specific preventive treatment over any others should be the basis to promote further research in this field, rather than to abandon it. For example, it is likely that a 'one-size-fits-all' treatment approach in CHR-P populations is not effective and that some treatments may work (only) for specific subgroups of patients. The implication here is that if testing treatments at group level (without stratification) produces negative results, we may potentially conclude that a treatment is broadly ineffective when it may, in fact, have efficacy for specific subgroups.

The failure of some studies, such as the NEURAPRO (omega-3) trial when tested in an unstratified CHR-P sample (McGorry *et al*, 2017), has naturally led to the suggestion that omega-3 might be more efficacious in those individuals who specifically have low levels of membrane fatty acids at baseline (Kane and Correll, 2017). This makes sense from a pathophysiological perspective—psychosis is a heterogeneous disorder with likely many different neurobiological 'paths' and risk factors (Radua *et al*, 2018), which may perturb an individual's neural circuitry in various (innumerable) ways and which ultimately presents as psychosis (Millan *et al*, 2016). Therefore, different individuals, despite all meeting CHR-P criteria, may respond differently to treatments depending on which neurobiological aberrations are contributing to the pathophysiological processes underlying their psychosis or CHR-P state. In the future, it may be possible to assess patients at first presentation for the presence of specific biomarkers which would indicate their likely response to specific treatments (or other future outcomes,

such as transition vs non-transition). This same method (depicted in **Figure 7–2** on page 171) could also be used to stratify individuals into research studies (e.g. low membrane fatty acids could indicate suitability for omega-3 trials) and may ultimately reveal that some of the previously tested treatments are effective in specific subgroups.

There are several lines of evidence to support the possibility that clinical heterogeneity may be affecting current (and previous) trials. First, some meta-analyses, including Papers 1 and 2 and a number of those in **Table 7–1** (on page 150), reported wide 95% confidence intervals for different treatments, indicating high levels of heterogeneity. Second, there is converging evidence that the CHR-P population is clinically heterogeneous. For example, there is high heterogeneity in the level of risk for psychosis across the three CHR-P subgroups: attenuated psychotic symptoms, brief and limited intermittent psychotic symptoms, and genetic risk and deterioration syndrome (Fusar-Poli *et al*, 2016b, 2016a, 2017a). In particular, those meeting the brief and limited intermittent psychotic symptoms criteria have a very high risk of developing persistent psychotic disorders. Such a heterogeneity calls for a revision of the CHR-P paradigm which, even at this early stage (in the absence of biomarkers), should include clinical stratification across these three subgroups. Ongoing international consortia such as PSYSCAN, PRONIA and NAPLS have already started delivering precision medicine tools for stratifying CHR-P individuals which may permit an individualised prediction of their outcomes and subgroup allocation for future clinical trials (NAPLS, 2018; PRONIA, 2018; PSYSCAN, 2018).

7.2.8. HOW SHOULD WE TREAT THE CHR-P STATE NOW?

Early intervention, prior to the onset of first-episode psychosis, is supported by more than 20 years of research in the CHR-P field (Yung *et al*, 2007). From a neurobiological perspective (Millan *et al*, 2016), it also makes sense to arrest pathophysiological processes as early as possible (Krystal and Anticevic, 2015; Lieberman *et al*, 2018) before more severe or enduring neural changes take place (that may be less amenable to later treatment). Keeping the critical developmental windows across the lifespan in mind (Marín, 2016), and given that we are currently not able to intervene in the so-called "pre-drome" (prior to overt symptoms) (Krystal and Anticevic, 2015), the CHR-P state—particularly in adolescence—would seem an apt time for intervention (Marín, 2016; Millan *et al*, 2016). The need for treatment during this stage is therefore well established. While we may not have biomarkers and definitive evidence for specific effective treatments for a number of years to come, it remains imperative to provide the

best evidence-based clinical care possible for CHR-P patients in the here and now. How, therefore, should we select interventions at the present time, given the state of the current evidence for (lack of) superiority of the different available treatments?

Overall, no reliable recommendations can be made regarding whether specific interventions (e.g. psychological interventions, medications, dietary interventions, needs-based interventions) are more or less effective compared to each other for the prevention of psychosis and reducing attenuated psychotic symptoms (Davies *et al*, 2018a, 2018b). Consequently, the safest approach is recommended, which would entail needs-based interventions and psychological interventions over antipsychotics, because the latter are not more efficacious than other options and have known side effects (Liu and Demjaha, 2013). In the absence of unequivocal evidence, the selection of interventions should also take into account the characteristics and needs of each individual. These may include patients' preferences (e.g. some patients may prefer talking therapies to any form of pharmacotherapy), social circumstances (e.g. needs-based interventions, which include housing/vocational support, may be suited to patients for whom these issues represent current stressors), nature of symptoms (e.g. CBT may be indicated for those presenting with cognitive biases), predicted risk (e.g. those presenting with brief and limited intermittent psychotic symptoms may need psychological treatments beyond needs-based interventions), or local service factors, such as the availability of each intervention (e.g. whether a waiting list is required for some treatments but not others) and staff training/competencies for delivering specific treatments.

Going forward, it will be essential to consult the results of forthcoming studies as they emerge. In this regard, a "living" network meta-analyses (discussed in section 7.4 below), defined as the updating of a (network) meta-analysis whenever a new eligible RCT becomes available, could provide new and specific evidence earlier than would be available through the updating of conventional meta-analysis (Nikolakopoulou *et al*, 2018).

7.3. EVIDENCE SYNTHESIS – STRENGTHS & LIMITATIONS

Strengths and limitations have been discussed in Papers 1 and 2. In addition, strengths of the two network meta-analyses presented here include the fact that the studies were pre-registered with detailed and specific analysis plans which included the node clustering definitions (Davies *et al*, 2017). However, even if network meta-analyses do

not require the "shoehorning" of treatments into inappropriate pairwise categories, there is still an element of clustering the specific interventions into nodes. We therefore tested the robustness of our findings to different clustering options in sensitivity analyses and found no material change to the results or conclusions. A major limitation of the field as a whole is that the evidence base is limited with still too few studies. However, our analyses are the most highly powered to date (by including the most comprehensive dataset available) and therefore provide the best treatment effect estimates currently available. Evaluating treatments on a binary outcome (transition to psychosis) as well as a continuous outcome (reduction of attenuated psychotic symptoms) also enabled us to show that our non-significant conclusions were not just secondary to low statistical power for testing the transition outcome (Davies *et al*, 2018a, 2018b). However, our measure of acceptability was crude and nonspecific (but pragmatic). One potential limitation in this field is that because allocating CHR-P patients to a pure "no treatment" condition is not considered ethical, relatively more "active" control conditions are used (such as needs-based interventions). It has been argued that these control conditions may be "too active" and therefore obscure significant treatment effects (Kane and Correll, 2017). However, ethical requirements mean that this is unlikely to change in future.

7.4. EVIDENCE SYNTHESIS – FUTURE DIRECTIONS

Individual participant data living meta-analyses

In addition to improved trial designs, including controlling sample risk enrichment (section 7.2.6), screening out of low-risk patients and clinical stratification (section 7.2.7), another precision medicine approach that could advance knowledge is the use of individual participant data network meta-analyses. The use of individual data as opposed to aggregate (study)-level data would allow (a) stratification of the efficacy of treatments across different confounders (including the three CHR-P subgroups), and (b) development of evidence-based prognostic algorithms to forecast the likelihood of treatment response at the individual subject level. It is expected that these individual participant data meta-analyses will identify specific subgroups of CHR-P individuals for whom current treatments (e.g. integrated psychological interventions, which had the largest—albeit nonsignificant—effect size OR=0.04) may already be effective. A relevant issue is that the future publication of a single study with robust evidence of efficacy may significantly change the level of evidence and conclusions regarding preventative treatments for psychosis. As discussed in the Introduction to Part 2, novel compounds for this patient population are under intense investigation and it is thus

expected that new results will be released over the next few years. Unfortunately, meta-analyses—even if based on individual participant data—are outdated as soon as new studies on the same topic emerge. Once published, only a minority of meta-analyses are then updated within two years of publication (Jadad *et al*, 1998). Such an inability to maintain recency may lead to significant inaccuracy and clinical practice which is not updated with evidence-based medicine. For example, by two years post-publication, 23% of non-updated meta-analyses will have failed to incorporate new evidence that would substantively change its conclusions (Shojania *et al*, 2007).

Cumulative meta-analysis, defined as updating a meta-analysis whenever a new eligible RCT becomes available, can be used to address this (Nikolakopoulou *et al*, 2018). However, the problem is that the median time taken for a primary study to be incorporated into a meta-analysis ranges from 2.5 to 6.5 years (Elliott *et al*, 2014). Thus, in 2014, "living" systematic reviews were proposed as a framework for continuously updating meta-analyses (Elliott *et al*, 2014). Living meta-analyses are particularly indicated when (a) the question to be addressed is essential to decision-making, (b) when there is uncertainty of the evidence, (c) when new information is likely to change the findings, and (d) when there is likely to be new evidence (Elliott *et al*, 2017). All of these conditions apply to the CHR-P field. A potential promising method for the CHR-P field would, then, be to combine the network meta-analyses presented in Papers 1 and 2 with living meta-analytical approaches over the coming years. A recent empirical study has demonstrated that prospectively planned living network meta-analyses produced strong evidence against the null hypothesis more often—and earlier—than conventional pairwise meta-analyses (Nikolakopoulou *et al*, 2018).

Finally, in addition to more sophisticated meta-analytic procedures, continued research into novel therapeutics is needed. This endeavour will be supported by increased understanding of the neurobiological mechanisms underlying psychosis risk and onset, which will allow us to develop treatments specifically targeted to precise neural processes rather than the current empirical attempts which are not (generally speaking) based on validated pathophysiological mechanisms (Millan *et al*, 2016). This would allow resources to be directed to the best-bet approaches and may expedite the search for effective preventive interventions. The growing use of experimental medicine approaches (as used in Part 2 of this thesis) are one way of assessing the potential therapeutic or mechanistic effects of potential novel treatments *in vivo* and can allow

the fast (and relatively low cost) assessment of a treatment's potential before embarking on later-phase clinical trials that may ultimately fail.

7.5. OXYTOCIN – FINDINGS IN CONTEXT & FUTURE RESEARCH

The purpose of experimental medicine studies is to (a) test a drug against a specific target to assess its likely therapeutic potential, and (b) provide support for go/no-go decisions regarding later (more resource-intensive) clinical trials. This is particularly advantageous for assessing the effects of oxytocin because despite the abundance of preclinical work (which shows promising physiological effects on blood volumes and glutamate), very few studies have tested these effects in humans (Aoki *et al*, 2015; Benner *et al*, 2018; Paloyelis *et al*, 2016) and none have tested this in CHR-P individuals. The two experiments presented in Papers 3 and 4 therefore allowed the first evaluation of oxytocin's effects in CHR-P individuals and have provided neurophysiological evidence of therapeutic potential. Without repeating the discussions from Papers 3 and 4 (Davies *et al*, 2019a, 2019b), the following section reviews the findings in light of key CHR-P mechanisms, factors impacting potential future translation of oxytocin into a therapy, and methodological strengths and limitations.

7.5.1. FINDINGS & PATHOPHYSIOLOGICAL MECHANISMS

One implication of our findings relates to mechanisms, and what the direction of our effects means in relation to established models of CHR-P pathophysiology. The model of CHR-P pathophysiology presented in Figure 1 in Papers 3 and 4 was used as the basis for our experimental hypotheses. The model posits that hypofunctioning NMDA receptors on GABAergic interneurons leads to disinhibition of pyramidal neurons, excess glutamate neurotransmission and increased perfusion, which together with downstream processes leads to attenuated psychotic symptoms and psychosis onset (Lieberman *et al*, 2018; Lisman *et al*, 2008; Modinos *et al*, 2015a; Schobel *et al*, 2013). Mechanistically, increased excitatory drive is mediated by increased extracellular glutamate, which increases metabolic demand and which therefore drives increased blood flow (Lecrux *et al*, 2019). Evidence suggests that dysfunction in these processes initially occurs in the hippocampal CA1 subregion, which spreads to the subiculum and extra-hippocampal regions (such as the frontal cortex) as frank psychosis develops (Schobel *et al*, 2009, 2013). The spreading functional pathology appears to be followed by structural changes—such as reduced hippocampal/cortical volumes (Ho *et al*, 2017a, 2017b)—which are likely less amenable to treatment, suggesting that targeting CA1 hyperperfusion or glutamate neurotransmission may have optimum therapeutic

potential. The neural circuitry involved in the above also provides numerous points (or processes) which could potentially become targets for therapeutic intervention, with the most likely targets being altered glutamate neurotransmission and hippocampal hyperperfusion (blood flow). For instance, one line of research may seek to directly normalise extracellular glutamate levels/neurotransmission (e.g. with n-acetylcysteine, lamotrigine or gabapentin), while another strategy may be to specifically target cerebral perfusion.

One issue is that because we cannot specifically measure *extracellular* glutamate levels in living humans, it can be difficult to interpret 1H-MRS results for novel pharmacotherapies, particularly when the findings are negative (such as in Paper 4). However, examining cerebral perfusion (which is measurable *in vivo*) offers a viable method for evaluating novel treatments in patients; glutamate/excitatory drive and perfusion are also closely linked, are both implicated in the same model of CHR-P pathophysiology and normalising perfusion is a goal in itself. The ultimate aim of drug (oxytocin) challenge in this scenario would, then, be to normalise hippocampal perfusion in CHR-P patients.

In line with this, in Papers 3 and 4 we used oxytocin with the aim of modulating the functioning of this neural circuitry. However, under our experimental conditions, oxytocin increased rather than decreased hippocampal perfusion. This would not seem to be the desired direction of effects, given that increased hippocampal perfusion has been associated with adverse outcomes in CHR-P individuals (such as non-remission or transition to psychosis) (Allen *et al*, 2016, 2018; Schobel *et al*, 2013). However, there are two reasons why the direction of effects does not immediately discount oxytocin as a candidate compound with potentially relevant neurophysiological effects. First, in the absence of a parallel group of healthy controls, we cannot infer which direction (an increase or decrease) would represent 'normalised' perfusion. We also do not know if the effects of oxytocin on hippocampal perfusion are specific to CHR-P patient groups—where we may expect altered perfusion at baseline—or whether this would also be found in healthy controls. Relatedly, this study was not powered (nor designed to assess) the effects of oxytocin on symptoms, and so we cannot infer whether increased perfusion is associated with improved or worsening symptomatology. Second, as discussed in Papers 3 and 4, the physiological effects of oxytocin are known to be highly dependent on the dose, route of administration and the duration of the sampling period (time between dosing and data collection) (Martins *et*

al, 2019; Quintana *et al*, 2016, 2017, 2019b; Spengler *et al*, 2017). Due to the cross-reactivity of oxytocin with vasopressin receptors—which is likely to occur with medium-to-high range doses (as used in the current study)—and given that vasopressin can produce diametrically opposite effects to oxytocin, e.g. vasopressin can be anxiogenic while oxytocin can be anxiolytic (Neumann and Landgraf, 2012), it is possible that lower doses of oxytocin will still engage the hippocampus but would decrease rather than increase perfusion. However, such an understanding can only come from future studies with the required design and sample size. While it would have been elegant to show that oxytocin modulates perfusion and glutamate/Glx concentrations in the same region, as reviewed in the Discussion in Paper 4 (and in section 7.6.2 on page 169 below), 1H-MRS may not be a sensitive enough gauge for evaluating neurotransmission within the neural circuits of interest, or oxytocin may simply not alter the concentrations of these neurochemicals.

7.5.2. COULD OXYTOCIN HAVE THERAPEUTIC EFFECTS?

As discussed in the Part 2 Introduction (section 4.3), no studies have yet assessed the effects of oxytocin on symptoms, functioning, preventing transition to psychosis, or social-emotion processing in CHR-P individuals. Therefore, in trying to understand the possible symptomatic effects, we are limited to previous studies in people with established psychotic disorders. However, there are numerous reasons why testing compounds in people with established psychotic disorders is not the same as in those at CHR-P. In line with the pathophysiological models and supporting evidence discussed above (section 7.5.1), CHR-P individuals may respond differently—and perhaps better—to certain medications because early neurobiological perturbations are perhaps (a) qualitatively different, and (b) more amenable to intervention (Lieberman *et al*, 2018; Millan *et al*, 2016; Schobel *et al*, 2013). Indeed, authors have described a need for illness stage-specific pharmacological intervention in psychosis and the CHR-P state (Krystal and Anticevic, 2015). CHR-P individuals are also (generally) antipsychotic naïve and lack illness chronicity. In addition, the ethical requirement for add-on studies in patients with established psychosis is avoided in CHR-P samples—a 'cleaner' estimate of efficacy can therefore be derived and smaller sample sizes can be used.

Evidence from established psychotic disorders

The early studies in patients with psychosis were promising in showing significant reductions across positive, negative, general and total symptoms (Feifel *et al*, 2010,

2015; Modabbernia *et al*, 2013; Pedersen *et al*, 2011). However, a series of negative trials have since followed (Dagani *et al*, 2016; Feifel *et al*, 2015; Jarskog *et al*, 2017) and recent meta-analyses have concluded that (a) oxytocin does not improve any aspect of schizophrenia psychopathology (positive, negative, general or total symptoms) (Williams and Bürkner, 2017), and (b) in terms of social cognition, benefits are likely to be found only for paradigms testing higher-order social cognition (Bürkner *et al*, 2017). However, there is some evidence that these recent negative results may be due to other factors rather than treatment failure per se. For example, an equivalence-testing study of intranasal oxytocin research found that of the 34 non-significant effects reported, 74% were due to data insensitivity rather than absence of an effect (Quintana, 2018). In fact, low study power has been an enduring issue in the oxytocin field and likely originated from the small, initial studies which showed large effect sizes for behavioural or questionnaire-style outcomes (Quintana, 2018; Walum *et al*, 2015). Such large effect sizes were likely false-positives but they set the tone for future studies, which did not account for the "winner's curse" phenomenon, and which were then subsequently underpowered (Button *et al*, 2013). In summary, the evidence we have from RCTs in psychosis have not shown robust or replicated benefits of oxytocin on any given outcomes. However, as per the reasons outlined above, this does not necessarily mean that oxytocin would be ineffective for people at CHR-P.

Acute vs long-term effects

Another factor to address is the effects of acute vs repeated administration and whether there are differing associated physiological and clinical outcomes. If, as has been indicated in forthcoming research (Martins *et al*, 2019), different devices for administering intranasal oxytocin also produce different neural effects, then future studies should ensure use of the optimal delivery method. This study also indicated that oxytocin's effects on subcortical perfusion—which is the primary target of interest for psychosis/CHR-P research—are present only ~15-35 mins post-administration (Martins *et al*, 2019). In future, if oxytocin's therapeutic effects are found to be related to modulation of subcortical function, then this time course may affect its viability as a treatment (because even twice/day dosing may not be sufficient). While in previous years alternative oxytocin receptor agonists—with longer half-lives—were being developed by multiple pharmaceutical companies (for childbirth), these have since all but ceased. However, a forthcoming study suggests that, when using the effects of oxytocin on amygdala reactivity to fearful faces and amygdala–prefrontal functional connectivity as an outcome, every-other day dosing was superior to daily dosing due to

the rapid decline in effects with daily treatment (preprint (Kou *et al*, 2018)). These factors will need to be investigated in future studies.

Going forward, larger RCTs that can assess clinical outcomes are required. Ideally, these would be preceded by—or in addition to—dose-finding studies, as well as studies testing different dosing regimens and methods of administration. Study outcomes could include reduction of attenuated psychotic symptoms, negative symptoms, higher-order social cognition and emotional processing. However, examination of these outcomes (especially attenuated psychotic symptoms) would require repeated dosing over longer durations so that symptom change can be reliably measured with validated CHR-P assessments (such as the CAARMS). Further longer-term trials (e.g. with a 12-month follow up) could also investigate potential preventative effects on transition.

7.6. OXYTOCIN – STRENGTHS & LIMITATIONS

7.6.1. ASL

Strengths of our ASL approach include the double-blind, placebo-controlled crossover design and the relatively large sample size for a within-subject neuroimaging study. Collecting data over two runs also allowed us to perform a combined analysis (i.e. with double the amount of data) in an attempt to increase the signal-to-noise ratio. However, there a number of important limitations. One is that the direction of the effect we observed in this study (increased perfusion) seems to be in the opposite direction to what one would predict would be therapeutic in these patients (i.e. a decrease) (Allen *et al*, 2016, 2018). This makes interpretation more difficult, but the key goal of our study was to show disease-target engagement—that oxytocin can alter perfusion in this target region. In addition, the dose effects of oxytocin are well known—the effect direction (increase vs decrease; or presence vs absence of effects) across indices of neural function (Spengler *et al*, 2017) and behavioural or symptomatic response (Kosaka *et al*, 2016; Quintana *et al*, 2017) is modulated by the given dose, likely due to cross-reactivity with vasopressin receptors (Galbusera *et al*, 2017; Heinrichs *et al*, 2009). Observing an increase in the current study does not, therefore, discount the possibility that different doses may engage the hippocampus but have effects in the opposite direction. A parallel healthy control group would enable better delineation of the direction of the effects and this remains an avenue for future research. One potential limitation is that we focused only on the hippocampus and omitted analyses into other regions strongly linked to oxytocinergic and social-emotional functioning, such as the amygdala (Zink and Meyer-Lindenberg, 2012). However, we opted for the

hippocampus because the *a priori* evidence for altered hippocampal perfusion in CHR-P individuals is strong and has been independently replicated (Allen *et al*, 2016, 2018), which is not the case for amygdala perfusion in CHR-P. Nevertheless, amygdala perfusion is altered in psychotic patients (Pinkham *et al*, 2015) and given the known effects of oxytocin on amygdala activation and functional connectivity (as discussed in section 4.3.3), such investigations remain avenues for future research. Another limitation (common to the both the ASL and 1H-MRS studies) was that we did not collect pre-post questionnaire data on key clinical outcomes, such as levels of attenuated psychotic symptoms. However, our study was designed and powered to detect neurophysiological effects and assessing efficacy for symptoms would require significantly larger sample sizes (and likely repeated dosing over time so that symptom change can be measured). Whether our neurophysiological findings translate into clinical efficacy therefore remains unknown and will need to be evaluated by future trials. Another limitation is that the regions-of-interest used in the current study were small (especially the hippocampal subregions), which is why we opted for a traditional region-of-interest approach (where one value is extracted for each patient in each condition, representing the mean perfusion across the total region-of-interest), rather than a voxel-wise region-of-interest analysis which can be affected by the cluster-level significance thresholds in SPM (when the clusters are small).

7.6.2. 1H-MRS

Strengths of our 1H-MRS approach included the double-blind, placebo-controlled crossover design and the relatively large sample size (although see the point about final sample size and power below), and the fact that none of our data were excluded due to poor quality spectra. 1H-MRS is also a relatively quick, non-invasive way to examine aspects of glutamatergic function *in vivo*. By conducting both creatine-scaled and voxel tissue content-corrected analyses, we reduced the possibility that our (ultimately negative) findings were due to methodological issues (e.g. changes in creatine itself, differing voxel placements leading to differing proportions of CSF/WM/GM). However, there are a number of important limitations. 1H-MRS cannot discriminate between intra- and extra-cellular metabolites and so provides total-voxel concentrations. As a result, while we have preclinical evidence for the pathophysiological model based on NMDAR hypofunction on GABAergic interneurons (leading to disinhibition of pyramidal cells; Figure 1 in Papers 3 and 4), and can measure 'overall' glutamatergic/Glx function using 1H-MRS as a proxy, we are not actually able to measure the microscale functioning of these neural circuits (i.e.

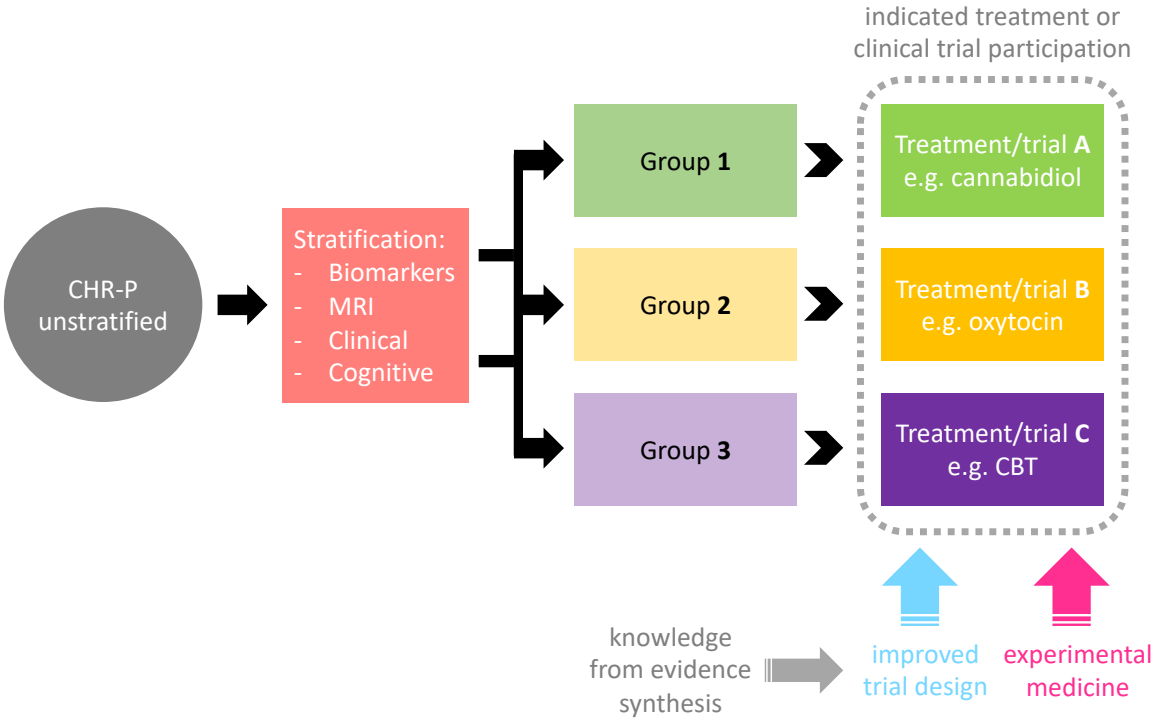
glutamatergic neurotransmission) in living human brain. This means that, in theory, oxytocin may have had an effect on the aforementioned neural circuits, restoring inhibition of pyramidal neurons and therefore decreasing glutamate signalling, but we are unable to detect such changes (which occur on the scale of micromolar (uM) concentrations) with 1H-MRS. However, previous studies using NMDAR antagonists (such as ketamine) (Kegeles *et al*, 2014; Kraguljac *et al*, 2017) or other compounds that alter glutamate release (such as n-acetylcysteine) (McQueen *et al*, 2018; Schmaal *et al*, 2012) have demonstrated changes in total-voxel 1H-MRS glutamate/Glx concentrations, which provides (indirect) evidence that modulation of neural circuit function *can* alter total-voxel metabolite concentrations. Therefore, it is conceivable that if oxytocin had acutely modulated glutamatergic neurotransmission, we may have expected to observe the effects using 1H-MRS. Nevertheless, this indirectness is a limitation and resolution of this issue would require the development of glutamatergic radiotracers. In addition to the limitations inherent in 1H-MRS, our updated power calculations suggested that our study was only powered to detect medium-to-large changes (Cohen's d ~0.55) in glutamate/Glx, which means we would have been unable to detect smaller—but potentially significant—effects. Increased power could be achieved in future by increasing the sample size. Another factor is that of the duration between dosing and 1H-MRS collection—it remains possible that we simply 'missed' neurochemical effects of oxytocin because our data sampling period was too delayed. Our acute dosing regimen also did not allow us to examine whether longer-term repeated dosing would have effects on metabolite concentrations.

7.7. INTEGRATING EVIDENCE SYNTHESIS & EXPERIMENTAL MEDICINE – TOWARDS THE PLATEAU OF KNOWLEDGE

As discussed throughout this thesis, it is hoped that ongoing research into the clinical and neurobiological stratification of CHR-P individuals will facilitate more successful clinical trials (as well as all other study types) across the field. For example, taking a precision medicine approach, stratifying individuals based on the presence vs absence of specific biomarkers is arguably the most promising way to reduce the heterogeneity within research samples. This will likely impact clinical trials (because specific drugs can be tested in those for whom it is indicated) and case-control studies of neural structure and function (because signal-to-noise will be increased in the group-level data due to reduced neurobiological heterogeneity).

Within the framework presented in **Figure 7–2** (below), evidence synthesis (as in Part 1) and experimental medicine (as in Part 2) are two important components—among a number of others—that together would help bring about a precision medicine approach to the CHR-P field (as well as improved research methodologies and hopefully, commensurate results). First of all, the knowledge from meta-analyses, such as those presented in Papers 1 and 2, provide critical insight into the current state of evidence, the most likely beneficial treatments for future investigation and can highlight issues that may undermine the success of RCTs—e.g. unstructured recruitment strategies leading to poor risk enrichment and low statistical power. This evidence can then be used to improve trial methodologies and increase the robustness of experimental findings. For example, treatments are more likely to show a signal if they are effective (e.g. because they are sufficiently powered), and if they show no signal of effectiveness, we can be more certain that this result is due to treatment failure rather than suboptimal study design.

Figure 7–2. How different research approaches complement each other towards precision psychiatry and improved treatments in the CHR-P field.



Second, prior to the types of studies examined in Part 1 of this thesis (RCTs for efficacy), a growing methodologic approach for use in drug development is experimental medicine. Given that ~75% and ~45% of novel compounds (with psychiatric indications) fail at phase II and III clinical trials, respectively (Hay *et al*,

2014; Hwang *et al*, 2016; Thomas *et al*, 2016), the screening out of the most likely failures (prior to phase II) is beneficial in terms of resources and time and can increase the chances of success for drugs that make it to later-phase trials. Experimental medicine also bridges preclinical work with human *in vivo* studies and allows exploration of possible mechanisms and novel therapeutic targets. In the CHR-P field, experimental medicine methods can be of particular value—in the absence of any validated pathophysiological mechanisms and biomarkers, we are currently in a time of desperate need for effective treatments, but we do not have a clear-cut 'assay' in humans that reliably identifies a drug as effective or ineffective. We are therefore proceeding with RCTs of pharmacological compounds that have a very high chance of failure. Experimental medicine studies can therefore provide critical evidence to support early go/no-go decisions for specific treatments. However, for the full potential of experimental medicine studies to be harnessed, reliable (even 'intermediate') biomarkers for each desired outcome in CHR-P samples is required. For example, if a novel therapeutic that effectively normalises hippocampal perfusion is also shown to decrease transition risk (or symptoms) over time, then reducing hippocampal perfusion becomes a target against which many novel (or repurposed) compounds can be tested against. Together then, it will likely be the combined contributions of evidence synthesis, experimental medicine and improved large-scale clinical trial methodologies that will ultimately pave the way to more effective treatments and improved outcomes for those at CHR-P.

7.8. CONCLUSIONS

In Part 1 of this thesis, I established that there is currently no evidence that any specific treatment is superior to any other treatment—including the lowest level needs-based intervention—in preventing transition, reducing attenuated psychotic symptoms or in acceptability in patients at CHR-P. These findings have contributed to knowledge because they were the first to challenge the prevailing view (NICE, 2014) that CBT is superior to all other treatments and should be offered to CHR-P patients as an evidence-based intervention. In Part 2 of this thesis, I established that oxytocin modulates resting hippocampal perfusion in CHR-P patients but does not appear to alter concentrations of glutamate (or Glx) in the hippocampus, ACC or thalamus when measured using 1H-MRS. These findings advance knowledge by demonstrating that oxytocin can engage one of the key pathophysiological targets associated with the onset of psychosis in patients at CHR-P, and therefore merits further investigation as a candidate novel treatment. Within this thesis, I have also argued that the prevention of psychosis from a CHR-P state has been, and should remain, the primary goal for interventional trials, refined and complemented by other clinically meaningful outcomes. However, as transition to psychosis is a relatively infrequent outcome, previous clinical trials in CHR-P populations have only been powered to detect large effect sizes for preventive treatments. It therefore remains possible that current treatments have preventative effects but of smaller magnitude. Controlling risk enrichment through recruitment and increasing sample sizes could help to mitigate this issue in future trials. Rather than give us cause to abandon the CHR-P field, the negative meta-analytic findings within this thesis (reflecting the 'stagnation of knowledge') should also promote innovative and collaborative research efforts, in line with the progressive and incremental nature of medical knowledge. Advancements will most likely be associated with the development of experimental therapeutics along with the ability to deconstruct the large heterogeneity within CHR-P populations. This would require the estimation of treatment-specific effect sizes through living individual-participant data meta-analyses, development of biomarkers or validated pathophysiological mechanisms underlying psychosis onset, and the use of experimental medicine approaches. The evidence-based challenges and proposed solutions addressed by this thesis can also inform the next generation of therapeutics research in the CHR-P field. It is hoped that these innovations will ultimately pave the way towards a future 'plateau of knowledge', effective preventative treatments and, most importantly, improved psychiatric, social and functional outcomes for patients and their families.

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